Weeds

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Weeds

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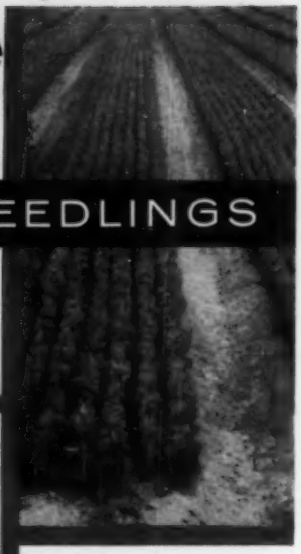

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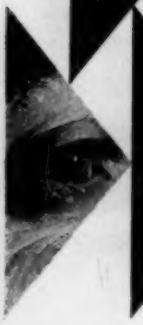

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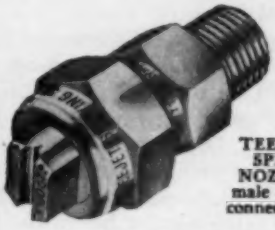

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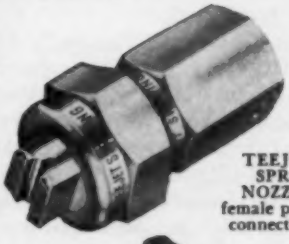

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Weeds

JOURNAL OF THE WEED SOCIETY OF AMERICA

VOLUME 7 APRIL 1959 NUMBER 2

Screening Procedure for Determining the Activity of Chemicals Against Imbibed Dormant Seeds[1]

STANLEY J. PIECZARKA and G. F. WARREN[2]

IN the field of weed control a great deal of attention is being given to the control of weeds after they germinate; in other words, pre- and post-emergence weed control. Control of dormant weed seeds in the soil has received little attention except in seedbed fumigation and in turf renovation. Many of the fumigant type materials were selected and screened for the control of nematodes and soil-inhabiting insects. It was discovered that some of them will control dormant seeds as observed by reduced weed stands as compared to non-treated areas.

Perhaps the lack of interest in the killing of dormant weed seeds is that no method for screening soil fumigants as to their toxicity to dormant seeds is available. There is also a lack of information as to what chemical or chemical groups are toxic to the dormant inbibed seed. In this paper soil fumigants or chemicals which control dormant imbibed seeds will be referred to as "seedicides". Information of this type is important as new seedicides could be synthesized in a systematic manner, incorporating the toxic chemical group into a stable molecule so that when applied to the soil, hydrolysis or microbial decomposition would release the toxicant, killing the dormant weed seeds.

Studies were undertaken in 1957 to develop a simple and efficient screening procedure for new seedicides and to determine the activity of selected organic chemicals to dormant imbibed seeds.

REVIEW OF LITERATURE

A literature review has revealed little direct information on the control of dormant imbibed seeds by soil fumigants or other chemicals. Stark (15) made an extensive study on the effect of chloropicrin on seeds which were held at different moisture levels and treated at different relative humidities. He concluded that the relative humidity during the time the seeds were exposed to chloropicrin vapor was of equal or more importance than the moisture content of the seeds. Workers in entomology, using a large number of fumigants,

[1]Journal Paper No. 1275, Purdue University, Agricultural Experiment Station.
[2]Research Assistant and Professor of Horticulture, respectively, Department of Horticulture, Purdue University, Lafayette, Indiana.

have reported that as the moisture content of the seed increases damage to the seed also increases (5, 6, 9, 11, 13). It has been postulated that the action of humidity is due principally to its effect on the moisture content of the seed coat. This assumes that the dry seed coat is impermeable to gases and when it absorbs moisture it becomes permeable, as fumigation of dry seeds produced little or no injury (9, 15).

Weed control has been very satisfactory with the use of methyl bromide and chloropicrin in seedbeds (1, 12, 18). Comparable results have been reported with the use of allyl alcohol and formaldehyde (1, 18).

Calcium cyanamide has been used for a number of years in seed bed and turf renovation. It is believed that the toxic principle to germinating seeds and young plants is hydrogen cyanamide from the hydrolysis of calcium cyanamide (1, 3, 4, 7, 10, 14). Moisture is necessary to initiate the reactions cyanamide undergoes.

MATERIALS AND METHODS

Inducing dormancy with low temperatures. In the development of a screening program for testing the effectiveness of various chemicals on dormant seeds, the seeds must be held in an imbibed dormant state to simulate the condition of weed seeds in the soil.

In order to determine the lowest temperature at which germination would occur seed were held for 2 weeks at the following temperature ranges: 32–36, 39–41, 42–46, 46–50, and 50–56° F. The seeds used were purslane *(Portulaca oleracea)*, pigweed *(Amaranthus retroflexus)*, tomato, muskmelon, sweet corn, and watermelon.

Petri dishes, 9 cm in diameter were used in which five pieces of filter paper were placed and moistened with 6 ml of a 0.02% KNO_3 solution. For the small-seeded species, 50 seeds were used per dish, for the large-seeded species, 25 seeds were used. At each temperature range five replicates were used of each seed tested. After the 2-week low temperature pretreatment the seeds were transferred to 77° F and germination counts were made every 2 days. A check was included which was germinated at 77° F without prior treatment.

Screening procedure employed. Seeds used in this study were purslane, pigweed, tomato, muskmelon, and watermelon. Seeds of purslane, pigweed, and tomato were held at 46–50° F and muskmelon and watermelon were held at 50–56° F. The seeds were handled as mentioned in the section on inducing dormancy with low temperatures except that they were held at the low temperatures for only 1 week. Four replicates of the petri dishes with covers removed were then placed in large glass containers, a volatile organic compound was injected at the rate of 0.88 ml, 1.76 ml, or 3.52 ml per container, placed for 24 hours at the pre-treatment temperature, vented for 2 to 4 hours, and then removed and germinated at room temperature (75 to 85° F). Germination counts were made every day for 10 days.

The containers used were 3-gallon specimen bottles. Rubber gas-

kets were placed inside the covers and two copper tubes (3/8 inch outside diameter) were soldered on the caps so that they extended 1 inch below and 2 inches above the cap. Tygon tubing was fitted over the copper tubing on the outside and closed with a screw type pinch clamp to obtain a tight seal. A piece of polyethylene with a hole in the middle was placed over the mouth of the jar and the cap screwed tight. This additional gasket helped to obtain a better seal between the cover and the mouth of the container.

A piece of cheesecloth was wrapped around one copper tube extending into the container and was tied in place (Figure 1). When

Figure 1. Close-up view of containers used in all tests. Note moist paper towels at the bottom to increase humidity and the gauze suspended from the copper tube to absorb the material being injected. The method in which the Petri dishes were stacked is also illustrated.

the material was injected into the container, it would first be absorbed on the cloth and then allowed to vaporize inside the container.

Forty two volatile organic compounds were tested. They were halogen, nitrogen, sulfur or sulfur-nitrogen derivatives of short chain aliphatic hydrocarbons. Some well-known soil fumigants were also included to determine the validity of the screening procedure.

The atmospheric treatment was modified for soil treatment so that new seedicides could be further screened and tested. Pigweed and tomato seed were used and handled in the same manner as in the atmospheric treatment except the seeds were planted in soil instead

of being placed on filter paper. Two soil types were used, a fine sandy soil and a muck which had been farmed for approximately 20 years. Four materials were tested at two rates, 0.88 and 3.52 ml per container.

RESULTS

Inducing dormancy with low temperatures. The germination percentages for some of the low temperature treatments are found in Table 1. Germination which occurred during the low temperature pretreatment was entered in the 14 day column. At the other low temperature ranges zero percent germination occurred during pretreatment. The rate of germination after placing seeds at 77° F is shown as accumulative percentages.

Table 1. Accumulative germination percentages of seeds at 77° F after holding 14 days at low temperatures.

Pre-treatment temperatures °F	Pct. germination during 14 days pre-treatment	Pct. germination at 77°F at various days after pre-treatment			
		2	4	6	8
Purslane					
Check	—	38	65	66	67
46–50	0	16	36	46	47
50–56	22	40	44	48	48
Pigweed					
Check	—	19	46	58	65
46–50	0	10	42	53	55
50–56	5	28	47	48	49
Tomato					
Check	—	0	91	93	93
46–50	0	82	89	90	91
50–56	44	88	91	91	91
Muskmelon					
Check	—	7	47	64	70
50–56	0	40	44	51	52
Watermelon					
Check	—	0	73	90	90
50–56	0	58	78	79	79
Sweet Corn					
Check	—	12	82	100	100
46–50	72	98	100	100	100

At temperatures below 50° F all the species except sweet corn failed to germinate. When held at 50–56° F, sweet corn germinated on the sixth day and purslane, pigweed, and tomatoes germinated on the 14th day. Muskmelon and watermelon had not germinated at this temperature range after 14 days. Visible swelling of tomato, muskmelon, and watermelon indicated that the seeds imbibed water at the low temperature. Seeds that were held at low temperatures required approximately the same length of time to germinate after moving them to 77° F as seeds which were moistened and germinated at 77° F without prior treatment.

In addition to the use of low temperatures, seeds were subjected to a high temperature treatment and to reduced oxygen tension as means of inducing dormancy. These methods did not prove satisfactory and were discontinued.

Screening procedure. The materials used in the atmospheric studies were given a toxicity rating (Table 2). Of the materials that

Table 2. Toxicity indexes of the materials used.

Material	Toxicity index[a]	Material	Toxicity index
Chloropicrin	10	1,1,2,2-Tetrachloroethane L	3
1,3-Dichloropropene	10	Carbon tetrachloride	2
Propionyl chloride	10	Bromoform	2
Bromine	10	n-Butyl bromide	2
Methyl bromide	10	HCN (H_2SO_4 generated)	2
Propionyl bromide	10	1,2-Dichloroethane	1
Methyl thiocyanate	10	Dichloromethane	1
Ethyl isothiocyanate	10	Ethyl disulfide	1
Ethylamine	10	Methyl mercaptan	1
n-Propylamine	10	Ethyl diamine	1
Ethyl isocyanate	10	Isopropyl *N*-phenylcarbamate	1
Allyl alcohol	10	Ethyl chloride	0
Formaldehyde	10	Trichloroethylene	0
Sodium *N*-methyldithio-carbamate dihydrate	10	Ethyl bromide	0
		n-Propyl chloride	0
1,2-Dibromoethane	9	Carbon disulfide	0
Ethyl thiocyanate	9	Ethyl mercaptan	0
Ethyl cyanoacetate	7	HCN (H_2O generated)	0
Dibromomethane	6	Methyl cyanide	0
Methyl disulfide	6	Propionitrile	0
Propyl mercaptan	5	Isopropyl *N*-(3-chlorophenyl)carbamate	0
Chloroform	3	Ethyl *N*,*N*-di-n-propylthiolcarbamate	0

[a]10 = 100% inhibition of germination, 0 = germination comparable to checks (no effect of chemical).

are being used as soil fumigants (chloropicrin, 1,3–dichloropropene, methyl bromide, allyl alcohol, formaldehyde, and sodium *N*-methyldithiocarbamate dihydrate) all gave 100 percent inhibition of germination with the five species used. Some of the chemicals, if used at higher rates, might possess greater toxicity to dormant seeds, whereas others might not be effective regardless of the concentration.

Table 3 gives a comparison of the atmospheric and soil treatment for pigweed and tomato seed with the four materials used in the soil treatment. In the atmospheric treatment, chloropicrin, 1,3–dichloropropene, and n-propylamine proved highly toxic to the dormant

Table 3. Germination percentages of pigweed and tomato in atmospheric and soil treatments.

Test plant	Chemical	Treatment					
		Atmosphere		Sandy loam		Muck	
		0.88 ml[a]	3.52 ml	0.88 ml	3.52 ml	0.88 ml	3.52 ml
Pigweed	Check	65	65	59	59	61	61
	Chloropicrin	0	0	0	0	35	1
	1,3-Dichloropropene	0	0	0	0	0	0
	n-Propylamine	0	0	48	0	43	47
	Ethyl thiocyanate	49	0	53	0	35	17
Tomato	Check	91	91	66	66	69	69
	Chloropicrin	0	0	0	0	13	0
	1,3-Dichloropropene	0	0	0	0	16	0
	n-Propylamine	10	1	30	0	43	43
	Ethyl thiocyanate	3	0	1	0	13	19

[a]Amount used per 3 gallon container.

seeds at both rates used whereas ethyl thiocyanate was toxic only at the high rate. The soil treatment illustrates the effect different soil types have on the toxicity of a compound. In the sandy soil all the materials were effective at the high rate while in the muck some of the compounds showed lower activity. Chloropicrin and 1,3–dichloropropene were effective at the high rates but n-propylamine and ethyl thiocyanate were not. This difference might be explained on the basis of differential adsorption by soil colloids.

DISCUSSION

The results in inducing dormancy in seeds by the use of low temperatures are in complete agreement with Kotowski's findings (8). At 11° C he obtained no germination of pepper, eggplant, cucumber, and muskmelon. The viability of the seeds mentioned was not affected at 11° C, 8° C, or 4° C as they all germinated when transferred after 35, 45, or 60 days to 30° C.

The use of low temperatures was selected for the tests for a number of reasons: (a) seeds could be moistened, placed at a low temperature, and left there for a week with no further attention, (b) the seeds required approximately the same length of time for germination when transferred to optimum conditions as did the checks, (c) the temperature ranges used closely approximate those of the soil in the spring or fall when fumigants are usually applied, and (d) results could be reproduced.

The screening procedure described could be simplified for a large scale operation by the following modifications: (a) use a single high rate for the initial test to determine if the chemical has toxicity; (b) use only 2 or 3 species, preferably weeds; (c) make germination counts less often, once a few days after treatment and again at the end of a predetermined time. Chemicals found active in this primary screening test could be further evaluated in more detailed experiments.

The inclusion of known soil fumigants confirmed the validity of the atmospheric treatment for testing new seedicides, as their toxicity index was ten. The soil treatment behaved as expected, in that the toxicity of some materials was reduced by adsorption or decomposition, especially in the muck.

An attempt to correlate boiling points, melting points, molecular weight, vapor pressure and thermodynamic activity with toxicity of the compounds examined proved unfruitful. Workers in entomology concluded that high vapor pressures were essential for toxicity (16, 17, 18). Vapor pressure is important in the distribution of the material in the soil but the material must also be toxic to the dormant imbibed seeds. Many of the compounds tested have high vapor pressures but low toxicity, while some had relatively low vapor pressure but were highly toxic. The fact that the vapor pressure is low is no indication of low toxicity or high vapor pressure of high toxicity. A compound of low vapor pressure may take a little longer to produce kills in the soil but remains in the soil for a longer period of time

at a lethal concentration. A material with too high a vapor pressure may be undesirable from the point of view that it will not remain in the soil for a period long enough to control dormant weed seeds.

The synthesis of a complex organic compound which is easily handled and decomposes releasing a simple compound which is toxic to the dormant seed would be desirable. An example of a material that is showing promise in the control of dormant seeds, is sodium *N*–methyldithiocarbamate dihydrate (SMOC) (Vapam or VPM). This soil fumigant is easy to handle end can be applied as a drench. Once in the soil, it decomposes releasing methyl isothiocyanate and the decomposition products kill the seeds.[3] The toxicity indexes of the materials used in this study clearly show that the iso- and thiocyanates are toxic to dormant imbibed seeds.

The materials that will be produced in the future as seedicides should: (a) have a toxic principle, (b) have adequate vapor pressure to move it through the soil, and (c) be rapidly destroyed or lost from the soil so that a crop can be planted in a reasonable time.

SUMMARY

Experiments were undertaken in 1957 to develop a screening program for testing new soil fumigants or seedicides against dormant seeds and to evaluate several volatile organic compounds as to their toxicity to dormant imbibed seeds.

1. To simulate the existence of dormant seeds in the soil, it was first necessary to establish a method of holding imbibed seeds in a dormant condition in the laboratory. The use of low temperature was the most successful of the methods tried.

2. Seed of purslane, pigweed, and tomato were placed in Petri dishes on moistened filter paper, and subjected to a temperature range of 46–50° F for 1 week. Seed of muskmelon and watermelon were handled in a similar manner, but kept at 50–56° F. These dishes, with covers removed were then placed in the 3 gallon bottle containers, each container sealed and a chemical introduced at the rates of 0.88, 1.76, and 3.52 ml per container. The containers were placed for 24 hours at the temperature used for holding the seed dormant. They were then exhausted for 2 to 4 hours with the aid of a fan, placed at room temperature and germination counts were made everyday for 10 days.

3. The atmospheric treatment for screening new seedicides proved to be valid as evidenced by the results obtained with known soil fumigants. It was then altered for testing new seedicides in the presence of soil. Two soil types and four materials were used. The results indicated that this technique could be successfully adapted for soil treatment.

4. A toxicity index of 42 organic compounds was obtained. The compounds were halogen, nitrogen, sulfur or sulfur-nitrogen derivatives of short chain aliphatic hydrocarbons.

[3]Benghiat, I. Personal correspondence. Stauffer Chemical Company, Chauncey, New York. 1957.

5. With the development of a technique that can be used for testing new seedicides and a partial listing of the degree of toxicity of organic compounds to dormant seeds, it is hoped that a systematic approach will be taken in the production of new seedicides. The ideal soil fumigant should be toxic to the dormant seed, have adequate vapor pressure to move through the soil, and move rapidly out of the soil so that a crop can be planted in a reasonable time.

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Selective Control of Wild Oats in Grain Crops by Use of Maleic Hydrazide[1]

A. C. Carder[2]

Wood (13) has estimated that 61,000,000 acres, or 60 per cent of the cropped land of the Great Plains region of North America, are infested with wild oats, *Avena fatua* L. The wide-spread seriousness of this weed and the fact that there is no cropping or tillage practice known for its ready control, point out the importance of finding a feasible chemical method.

The use of MH (maleic hydrazide) for the control of wild oats was investigated by Knowles (8) at Ottawa in 1952. It was found that this chemical applied at certain stages of growth would render the seed sterile. Since wild oats and grain crops are not usually in similar stages of phasic development at the same time, the object was to discover if it was possible to devitalize the wild oat kernels without injury to the cultivated crops. Although the wild oat plants themselves are not killed by MH, one might be able to eliminate wild oat seeds from the soil if one could prevent the production of fertile seed. The procedure would have to be continued over a number of years and the seeds already in the soil "grown out".

Subsequent work was conducted at Experimental Farms at Ottawa, Ont.; Beaverlodge, Alta.; Lacombe, Alta.; Regina, Sask.; Indian Head, Sask.; Melfort, Sask.; and Brandon, Man. Parts of this work have been published in abstract form (1, 2, 3, 5, 6, 7, 10, 11, 12). Ebell and Corns (4) at the University of Alberta reported briefly on work, the results of which are in general agreement with those of the above investigations.

In 1955, at a staff meeting of members of the Field Husbandry Division of the Experimental Farms Service, a committee was appointed to assemble the available information on the use of MH for the control of wild oats. The author was given the responsibility of reporting this information. This paper presents the results.

Materials and Methods

Since 1953, the procedure employed by the various workers at the different stations has been quite uniform. The objectives were to determine: 1) the best time to apply MH to obtain greatest injury to the wild oats and least to the cultivated crop, 2) the most economical rate of application, and 3) whether wild oat populations decreased if the chemical was applied annually.

In each experiment parallel strips of barley, wheat, flax and cultivated oats were drilled at the usual time into an area known to be heavily infested with wild oats. To secure the greatest possible difference between the phasic development of cultivated grains and of wild oats, early maturing, popular varieties of cereals were frequent-

[1]Contribution from the Experimental Farms Service, Ottawa, Canada.

[2]Agronomist, Field Husbandry Division, Experimental Farm, Beaverlodge, Alberta.

ly used. By seeding on a well and freshly prepared seed-bed, a considerable degree of uniformity among wild oat plants was obtained. The MH was applied crosswise to the drilled strips at different times and rates with low-volume plot sprayers. Replication varied at the different locations from two to six times.

Formulation MH–30 which contained no wetting agent was used for most of the early trials. However, a wetting agent such as Dreft, Triton or Orvus was added as directed (14). Later, in 1954, a wetting agent was incorporated by the manufacturers (15), and it was in this form that the product was used for all the more extensive experiments. MH–30 is a solution of the diethanolamine salt of MH and contains 30 per cent active ingredient by weight or 3.0 pounds MH per gallon. Hereafter, rates are expressed as active ingredient.

Since time of application of MH was found to be critical (2, 8) treatments were spaced a day or two apart whenever application time was the factor being studied.

Rates of MH from 8 to 24 oz/A were used in the earlier trials. Later, much lighter dosages, ranging from 2 to 8 oz/A, were investigated. In all tests the MH was applied in aqueous solution at rates of from 5 to 10 gal/A.

Most of the germination trials to test the devitalizing effect of the chemical on the seed of the cultivated crop and the wild oats were conducted through the cooperation of the Division of Plant Products, particularly the District Seed Laboratories at Calgary, Winnipeg and Ottawa. The germinability of the seed of the cultivated cereals was determined according to standard procedure, but that of wild oats was tested by special technique[3] to ensure a high degree of breakage of dormancy.

Certain trials were designed for the perennial application of MH. These involved a simple crop rotation such as two crops of an early variety of barley followed by summerfallow, or of barley and flax followed by fallow. The chemical was applied to one half the seeded area at a time when it was considered that the greatest amount of selectivity between the crop and wild oats would be obtained. Rate of application was 8 oz/A in some trials, while in others it was 16. Degree of wild oat infestation was determined by plant count on the treated and non-treated areas in the years subsequent to each MH application. In the fallow year the wild oats were controlled by tillage.

Quality tests were made on samples of seed treated and untreated with MH. Wheat was tested for protein content and milling and baking quality, flax for oil content and iodine number, and barley for malting quality.

EXPERIMENTAL RESULTS

Beaverlodge trials: These were conducted in 1953 and 1954 and involved Olli barley, Redwing flax, Saunders wheat and Larain oats. All are early varieties and are popular in the Peace River region except Larain oats. Maleic hydrazide was applied at rates of 8, 16,

[3]Lewis, N. G. Analyst, District Seed Testing Laboratory, Calgary, Alberta. Personal communication, 1954.

and 24 oz/A. Ten applications were made in 1953 and 6 in 1954 with 2 to 4 days between treatments. Samples of seed from the wild oats and cultivated crops treated with the chemical were collected to determine its effect on germination and yield.

To assist in determining when the wild oat plant was most susceptible to MH treatment, its phasic development was divided into 25 stages ranging from the shot blade stage to the hard dough or ripe stage. In Figures 1, 2, 3 and 4, treatment time is depicted on this basis. Because the growth stages of the cultivated crops do not coincide with those of wild oats their phasic development is not related to this scale.

Only the effects of MH when applied at 8 oz/A are given in Figure 1 which presents the Beaverlodge data, since this rate proved the best for all practical purposes and the results from the heavier applications are closely similar. The data indicate that the devitalizing effect of MH on the seed of wild oats became pronounced when the plants were in the late anthesis to kernel forming stage (Stage 10) and remained so to late milk (Stage 17). The most susceptible period varied somewhat according to season. Thus, in 1953 it extended from kernel forming (Stage 11) to milk (Stage 15), while in 1954 it centred at the milk to late milk stage and late milk (Stages 16 and 17). The data also show that at the time the seed of wild oats was strongly affected that of barley was not. A considerable degree of selectivity in the effect of MH was obtained between the seed of wild oats and flax in 1953, but this condition was not repeated in 1954. No possibility of selection is shown to exist between the seed of wild oats and that of wheat or cultivated oats.

Ottawa trials: Data from only the most extensive trial are presented. In this instance three rates of MH, 4, 8 and 16 oz/A, were used, but since they produced similar effects only the results of the chemical when applied at 8 oz/A are given in Figure 2. These results are essentially the same as those obtained at Beaverlodge. The seed of wild oats was most susceptible to MH at the kernel forming stage (Stage 11), while the seed of barley was practically unaffected at this time indicating a high degree of selectivity between the two species. However, unlike the Beaverlodge results, good selectivity was also manifested between wild oats and flax and between wild oats and cultivated oats.

Other trials in Western Canada: A test at Lacombe involved 6, 8 and 12 oz/A of MH applied to wild oats in Olli barley, but for reasons pointed out above only the results from the 8 oz/A rate are presented (Figure 2). It is again indicated that the seed of wild oats is markedly devitalized by MH if applied at the early milk to milk stages (Stages 13 and 15). The effect of the chemical on germination of seed borne by early panicles was not much different from that borne by panicles in a later stage of development. Some reduction in germinability of the seed of barley occurred when the plants were treated at the time the wild oats were highly vulnerable. Selectivity, therefore, was not complete.

At Regina in 1954, a 16 oz/A rate of MH was applied to wild oats in Vantage barley, Rocket flax, Selkirk wheat, and Rodney oats at

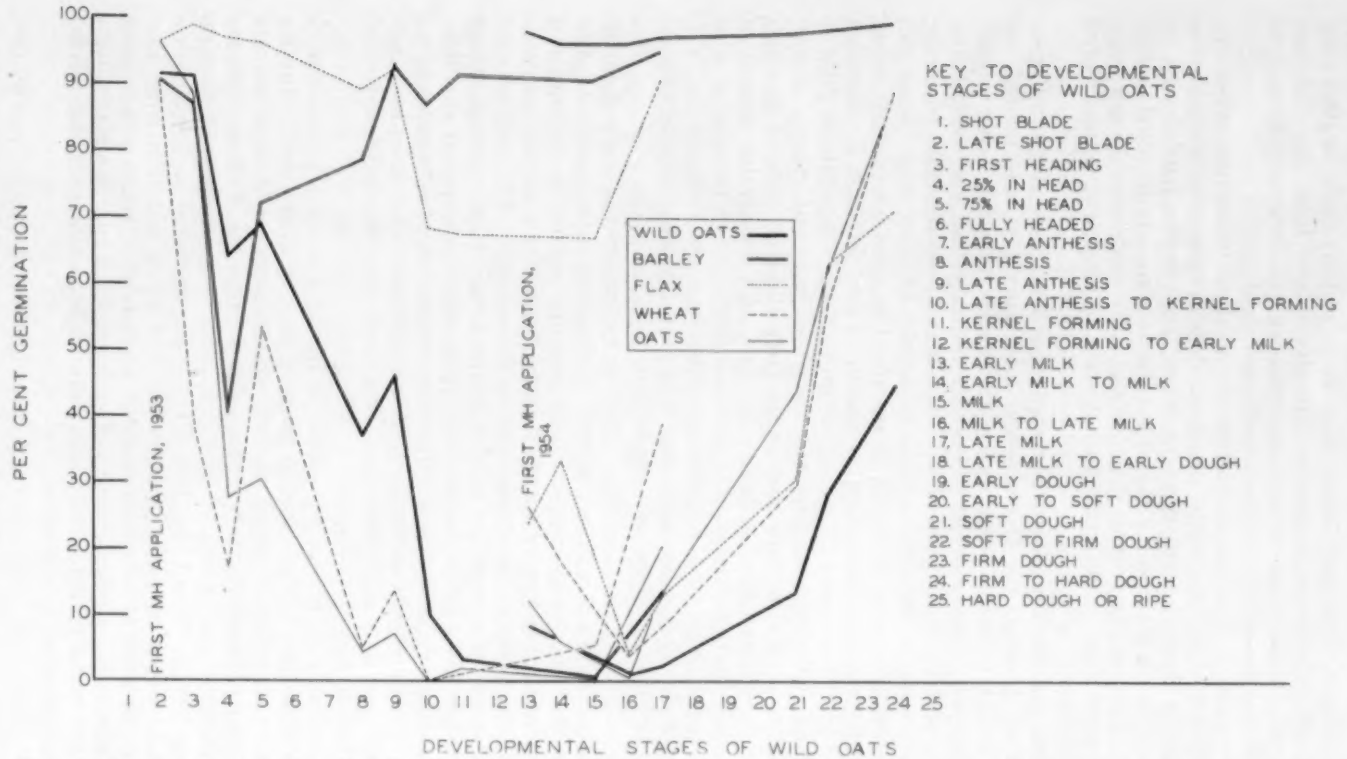

Figure 1. Percentage normal germination of seed of wild oats and grain crops following treatment at different times with 8 oz/A of MH. Beaverlodge, 1953 and 1954.

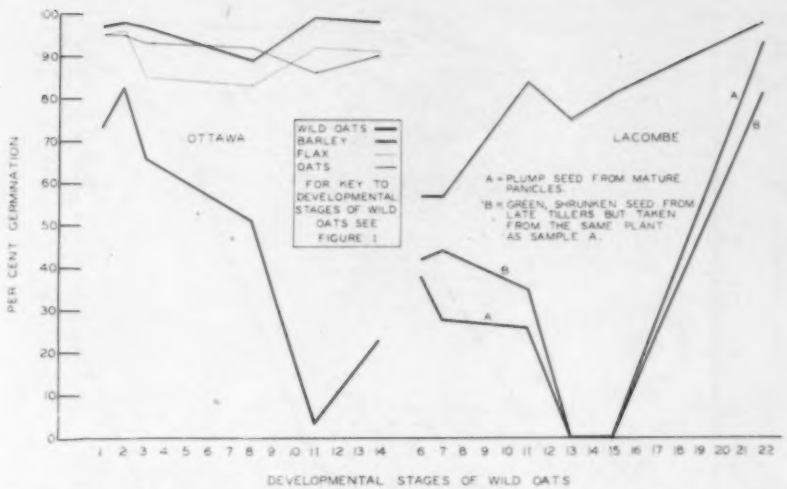
PER CENT GERMINATION
OTTAWA
WILD OATS
BARLEY
FLAX
OATS
FOR KEY TO DEVELOPMENTAL STAGES OF WILD OATS SEE FIGURE 1
LACOMBE
A = PLUMP SEED FROM MATURE PANICLES.
B = GREEN, SHRUNKEN SEED FROM LATE TILLERS BUT TAKEN FROM THE SAME PLANT AS SAMPLE A.
DEVELOPMENTAL STAGES OF WILD OATS

Figure 2. **Percentage normal germination of seed of wild oats and grain crops following treatment at different times with 8 oz/A of MH. Ottawa and Lacombe, 1955.**

different growth stages. The results at Regina are much the same as elsewhere. As shown by Figure 3, the seed of wild oats was almost totally devitalized by MH when applied to the plant in the early milk to milk stages (Stages 14 and 15). No marked selectivity, however, was obtained between the wild oats and the cultivated grains in which they grew. The reason for this was that the phasic development of the crop varieties corresponded closely to that of the wild oats. Earlier maturing varieties would have been preferable. Titan barley, Marine flax and Ajax oats mature several days earlier than the varieties which were under test. Apparently there is no good substitute for Selkirk wheat in that area.

A test conducted at Melfort in the same year but using Montcalm barley, Redwing flax and Thatcher wheat gave results similar to those of the Regina trial (Figure 3). Again, earlier maturing varieties than those used would have been preferable.

The results of a trial at Brandon using Vantage barley, Marine flax and Selkirk wheat are presented in Figure 4. These results are of interest since they differ from those of other trials. Only partial devitalization of the seed of wild oats occurred when treated at the early milk stage (Stage 13), but when treated as late as firm dough (Stage 23) total devitalization was obtained. The data show that a high degree of selectivity was possible between wild oats and barley, but the time of application was not consistent with that indicated by tests conducted elsewhere. No marked degree of selectivity was obtained between wild oats and flax or wheat at Brandon.

Tests at Indian Head in 1955 indicated that when wild oats were treated at the soft dough stage with up to 8 oz/A of MH only partial devitalization occurred. The germination of Raja flax was

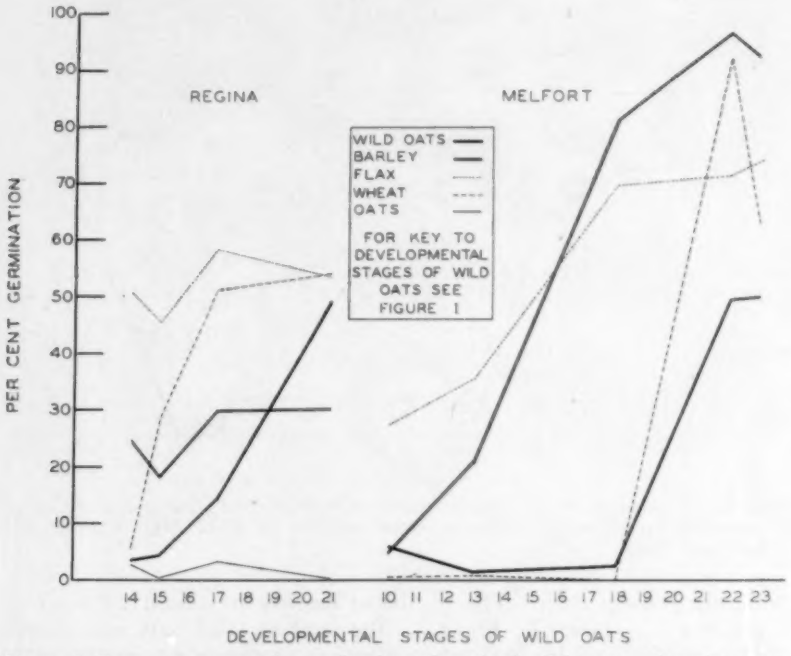

Figure 3. Percentage normal germination of seed of wild oats and grain crops following treatment at different times with 16 oz/A of MH. Regina and Melfort, 1954.

not affected by the treatments which were carried out when the bolls were well formed.

Maleic hydrazide was used to treat wild oats in a field of Laxton's Progress peas at Creston, British Columbia. The chemical was applied at the rate of 8 oz/A by airplane a few days prior to swathing of the peas for seed. The majority of the wild oats were at the milk stage at this time. The germinability of the peas was reduced from 98.2 to 85.5 percent by treatment, that of wild oats from 89.8 to 0.8.

Perennial trials: At Beaverlodge in the spring of 1954 two series of plots were selected for perennial trials: One series of untreated plots on which the wild oats had produced seed with a germination of 87 percent, the other where the germination of the wild oats seed had been reduced by MH treatment to less than 3 percent. Counts of seedling wild oats showed that the untreated plots averaged 155 plants to the square foot; the treated plots 31. Similar results were obtained at Lacombe. Plots untreated in 1955 contained 166 plants per square yard in the spring of 1956, while plots, effectively treated with MH, supported 56 plants per square yard.

To prove the reliability of the MH method it must be shown that control can be obtained by repeating the treatments over several years. Results of a rotation experiment at Brandon in which

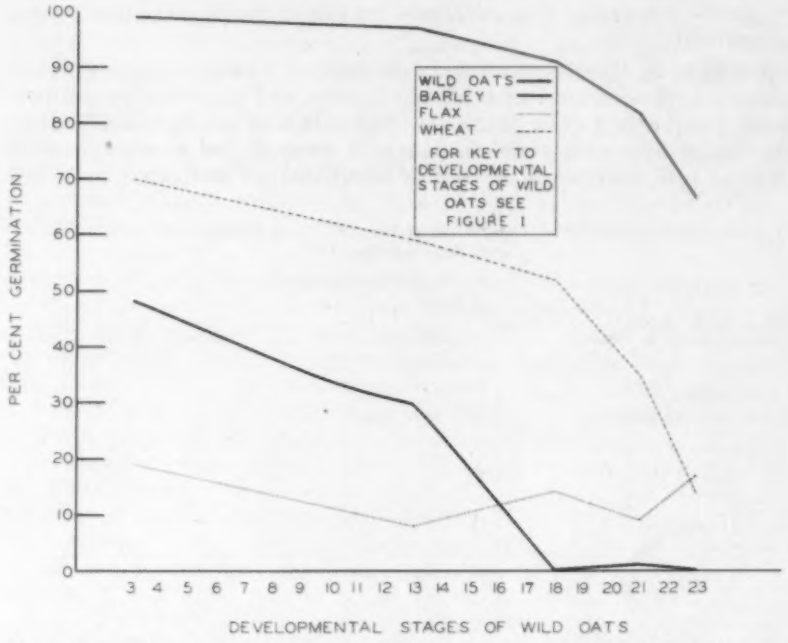

Figure 4. Percentage normal germination of seed of wild oats and grain crops following treatment at different times with 16 oz/A of MH. Brandon, 1955.

Table 1. Reduction of wild oat infestation by effective MH treatment. Brandon, 1955–57.

Crop	Year culm count taken	No. of wild oat culms per sq. yard				Wild oat culms on treated area as percent of untreated
		Treated in 1955	Untreated	Treated in 1955 & 1956	Untreated	
Barley	1956	49	288			17.0
	1957			4.9	36.5	13.4
Flax	1956	40	338			11.8
	1957			18.8	64.3	29.2

plots were treated with one or two applications of MH and compared with untreated plots are shown in Table 1. The data in the right hand column show that a marked reduction in wild oat infestation was obtained with MH. These figures also indicate that no additional reduction was obtained with a second application. However, despite this high degree of control, the number of wild oat culms in the treated areas was still too high to consider the control as being effective. The culm counts show a marked reduction in infestation from 1956 to 1957. Since this decrease is equally as great in the untreated plots as in the treated, the difference is undoubtedly due to seasonal conditions which caused many more wild oats to germinate in 1956 than in 1957.

Trials involving the perennial application of MH are being continued.

Quality tests: Results of analyses on samples of seed of spring wheat treated and untreated with MH at Regina and Beaverlodge are presented in Table 2. The data show that MH had no appreciable effect on quality. Quality tests on flax seed treated and untreated with MH at 4, 6 and 8 oz/A at Indian Head in 1955 indicated that seed

Table 2. Effect of MH at 8 oz/A on quality of spring wheat in trials at Regina and Beaverlodge, 1955.

Stage of wheat at time of treatment	Wt. per bushel lb	Protein pct	Flour yield pct	Flour pigment ppm	Water absorption pct	Loaf volume cc	Crumb texture 1–10	Crumb color 1–10
Selkirk wheat—Regina								
Kernel forming to early milk	61.1	13.9	71.9	1.6	60.4	760	6.5	7.0
Early milk	62.0	13.7	71.9	1.5	60.4	790	6.8	7.0
Milk	62.3	13.3	71.5	1.5	60.0	785	6.5	7.0
Soft dough	61.7	13.3	73.0	1.5	60.7	755	7.0	7.0
Firm dough	62.2	14.0	73.2	1.5	60.4	775	7.0	7.0
Firm dough	62.6	13.2	72.3	1.5	60.2	775	7.0	7.3
Untreated check	61.8	13.4	72.0	1.6	60.9	765	7.0	7.0
Saunders wheat—Beaverlodge								
Firm dough	64.3	12.7	67.4	1.5	65.2	610	4.0	2.0
Untreated check	65.2	12.9	65.2	1.6	64.9	575	4.0	3.5

oil iodine number (Wijs) and percentage oil content were also unaffected by MH treatment. However, quality analyses on Parkland barley, a malting variety, from plots at Brandon showed that in this instance MH treatment did have an effect. Data from these analyses are shown in Table 3. Here, MH treatment seemed to have increased

Table 3. Effect of MH at 16 oz/A on quality of malting barley. Brandon, 1955.

Treatment	Heavy grade pct	Kernel weight gm	Nitrogen pct	Malt extract pct	Wort nitrogen pct	Sacch. activity °L
Sprayed sample	64.0	31.6	2.21	1.28	73.9	170
Untreated check	51.6	29.3	2.15	1.26	73.4	125

yield of heavy grade barley without affecting to any extent the other properties except saccharifying activity. The increase in saccharifying activity was relatively great and indicated that certain enzyme systems were affected to a marked degree. Excess diastatic activity is regarded with apprehension by the maltsters as it leads to problems in malting and brewing.

DISCUSSION

The data from the various trials indicate that a high degree of kernel devitalization was obtained at every instance that MH was applied to wild oats at the milk stage. Under certain conditions this

highly vulnerable period was very short, while under other conditions it lasted through several developmental stages of the plant. For instance, at Beaverlodge in 1953 the period extended from the kernel forming to the milk stage, while at Melfort in 1954 it extended from the late anthesis to the early dough stage. In 1955 at Brandon, germinability was completely to almost completely destroyed when the rate of 16 oz/A was applied to wild oats from the late milk to the firm dough stage. On the other hand, at Ottawa, the highly vulnerable period was apparently very short, being confined to the kernel forming period.

Barley proved generally less vulnerable to MH treatment than the other grain crops. This and the fact that its phasic development was usually quite different from that of wild oats provided opportunity for the selective devitalization of seed when compared to wild oats. Olli barley proved noteworthy in this regard. In some instances, particularly at Ottawa, selectivity was obtained between wild oats and flax. In most cases, however, the phasic development of the two species of plants was not sufficiently different to insure this eventuality. The response of wheat and cultivated oats to MH was essentially the same as that of wild oats. Moreover, there seems little possibility that varieties of these crops are available whose phasic development is sufficiently different from wild oats to permit any degree of selectivity.

Attempts were made at a number of points to determine the optimum rate for succesful MH treatment. The Ottawa experiments indicated that this rate lay between 4 and 8 oz/A. In western Canada dosages of 2, 4, 6, and 8 oz/A and up were used. At Brandon almost complete devitalization of wild oat kernels was obtained with as little as 4 oz/A. In other trials 6 and 8 oz/A gave this degree of devitalization, while 4 oz/A did not. In a test at Lacombe almost complete devitalization of the wild oat kernel was achieved with 2 oz/A. Taken as a whole, the data indicate that 8 oz/A and certainly not less than 6 are necessary for dependable results. Rates greater than 8 oz/A seem unnecessary if the chemical is applied when the wild oats are at a vulnerable stage.

Although yields are not reported in this paper, investigations at Beaverlodge showed that despite the pronounced devitalizing effect of MH on wild oats it had no effect on yield of seed. The effect of treatment on yield of seed of cultivated crops was determined both at Beaverlodge and Ottawa. Results are in agreement and indicate that the yield of barley was not reduced by any treatment, but that of flax, wheat and cultivated oats was lowered when plants were treated at their earlier growth stages. Later treatments, however, had no effect on the yield of these last three crops. Therefore, it would seem possible to obtain normal yields, while destroying the germination of wild oats. The wheat and cultivated oats, however, would not be suitable for seed as their germination would be seriously impaired.

Unfortunately, there are not enough data available to draw final conclusions on the feasibility of this method of controlling wild oats

when MH is applied annually over a period of 3 or 4 years. Preliminary investigations suggest that marked reductions in the number of wild oats can be expected in the treated areas after MH treatments. It appears, however, that these reductions will not be great enough to be of practical significance, even if treatment is done consecutively over a period of 2 or 3 years. In the final evaluation of this control measure, other factors such as the critical nature of timing the application and the length of time required before results become obvious will have to be considered.

To determine if MH treatment results in induced dormancy or actual death of the wild oat kernel, samples of wild oats from plants treated with MH at Beaverlodge were sent to Ebell[4] for tetrazolium and embryonic tests. This seed showed strong activity of enzymes capable of reducing 2,3,5,6–triphenyl tetrazolium chloride (tetrazolium). This suggests that the seeds are still living but somehow incapable of normal germination. Observations suggested that the MH treatment prevented seed germination by interference with embryo development but permitted normal endosperm formation. These results agree with those of Mericle et al (9) who showed that the embryo of the seed of cultivated oats was markedly affected by MH when applied shortly after fertilization.

Since the wild oats were not actually killed by the MH, tests were conducted to discover whether or not the effect on germination decreased with time. The germinability of 216 samples of seed from wild oat plants treated with MH in 1953 at Beaverlodge was compared to that of seed obtained 2 years later from the same samples. Both sets of germination tests were conducted under identical conditions and technique. The results showed that in 199 of the comparisons there was no significant difference in germinability, while 9 of the remainder showed a significant increase and 8 a decrease.[5]

A series of 128 germination tests, conducted as described above for wild oats, was made on the seed of the cultivated crops. The results indicated that 118 of the comparisons showed no significant difference in germinability, while 3 showed a significant increase and 7 a significant decrease. Therefore, as with wild oats, there is apparently no dissipation of the effect of the chemical over 2 years. In these tests, untreated seed showed no decrease in viability during this time.

Tests have been made on samples of wheat, barley and flax collected at Beaverlodge from plants treated with various rates of MH–30 to determine what amount, if any, of the chemical entered into the seed. Although sizeable residues of MH were found, toxicological tests have shown that if recommended rates of the chemical are used the residues are within the tolerance limit established for edible crops.[6]

[4]Ebell, L. F. Department of Plant Science, University of Alberta, Edmonton. Personal communication, 1955.

[5]$P = .025$; size of sample = 100 seeds.

[6]Lougheed, A. W. Naugatuck Chemicals, Elmira, Ontario. Personal communication, 1956 and 1957.

SUMMARY

Use of MH for controlling wild oats consists of the application of the chemical to grain crops when the wild oats are in the milk stage, with the object of devitalizing the wild oat kernel without injury to the seed of the cultivated crop. The feasibility of the method was investigated at nine different points across Canada from 1952 to 1957.

From the results it is concluded that:

1. MH applied to wild oats or grain crops at certain times after fertilization devitalized the seed subsequently produced. Under most conditions this susceptible period was the milk stage. At Brandon the dough stage was apparently the most susceptible stage under the local conditions.
2. Eight ounces of MH per acre were sufficient for effective results. Properly timed and applied, this rate reduced germinability of wild oats to less than 5 per cent.
3. Under conditions of rapid development, the highly vulnerable period of wild oats to MH was very short, sometimes as little as 3 to 5 days.
4. Even at their most vulnerable stage, barley in particular and to some extent flax were affected less by MH than oats, wild oats, and wheat.
5. The phasic development of barley, especially on early maturing varieties, was sufficiently different from that of wild oats to permit selective devitalization. Under some conditions certain varieties of flax and peas may also permit this but it was not feasible with oats and wheat. No matter what crop is involved, however, there will always be a danger of injury to the grain so that germination would have to be tested if the grain is to be used for seed.
6. Successful devitalization of wild oats by MH for two successive years in heavily polluted fields brought about appreciable reduction in stand of wild oats. However, such treatments did not reduce the infestation to a practical level of control.
7. The yield of grain of the cultivated crops was not reduced by MH if it was applied after the crops had reached the early dough stage. Moreover, the grain from crops so treated was non-toxic and could be used as feed.
8. Milling and baking qualities of wheat were not affected by MH treatments, nor was iodine number and percentage of oil content of flax. The malting qualities of barley were affected.

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Cytological Effects of CDAA and IPC on Germinating Barley and Peas[1]

D. T. Canvin[2] and G. Friesen[3]

In recent years weed research workers have begun to investigate the possibility of using herbicides for the selective control of wild oats (*Avena fatua* L.) in field crops. One of the first herbicides to show promise for the control of wild oats was IPC (isopropyl *N*–phenylcarbamate). Although this herbicide was effective on wild oats, only a few broad-leafed crops proved tolerant to it. More recently, another herbicide, CDAA (2–chloro–*N*,*N*–diallylacetamide), has been found to be effective in controlling wild oats without damaging corn and soybeans, and several field tests indicate that it may be used successfully in flax. To be effective both herbicides must be applied as pre-emergence or pre-planting treatments. They kill wild oats as the wild oats germinate, and consequently must be placed in the vicinity of the germinating seed if a good job of killing is to be done.

A more thorough knowledge of the toxic effects of CDAA and IPC on germinating seeds would aid in the full development of their herbicidal potentialities. To further determine the toxic mechanism of these herbicides the cytological effects on germinating seeds were investigated.

Review of Literature

Ennis (5) examined barley roots cytologically and reported increased mitotic activity with a larger number of metaphases and fewer anaphases in germinating seeds treated with IPC over that found in untreated plants. Multipolar spindles occurred with chromosomes separating into more than two groups. In studying the effects of IPC on *Avena*, Ennis (4) reported that anaphase bridges, blocked metaphases, nuclear fragments, binucleate cells and vesiculate nuclei were apparent. He suggested that IPC probably acts by impairing or inhibiting the action of spindle fibres.

Doxey (3) observed that chromatids failed to separate at metaphase and that anaphase movement was inhibited in rye plants treated with IPC. He considered IPC to have a direct chemical action on cell division.

Derenne (2) studied the cytological effects of IPC on barley and onion and observed cells where polyploid restitution nuclei were formed. In other cells unequal anaphase division resulted in the

[1]This material is taken from the senior author's thesis presented to the Faculty of Graduate Studies of the University of Manitoba in 1957 in partial fulfilment of the requirements for the degree Master of Science.

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formation of cells with micro-nuclei. He concluded that the chemical acted as a mitotic poison and directly affected nuclear division.

In an attempt to determine the mode of action of CDAA, Jaworski (6) treated germinating seeds of wheat and rye grass with a 10 ppm solution. He suggested that CDAA acts on susceptible plants by inhibiting sulfhydryl enzymes and possibly oxidative phosphorylation.

MATERIALS AND METHODS

The cytological effects of CDAA and IPC were determined on Parkland barley and Chancellor peas. These crops were chosen because of their low chromosome number. Seeds were germinated in petri plates containing 5 ml of the desired concentration of chemical (active ingredient), and allowed to germinate for 96 hours at room temperature. Control petri plates contained 5 ml of distilled water. The root tips were collected at the end of 96 hours and fixed in Navashin fluid (1) for 3 hours. After a thorough washing they were dehydrated and embedded in paraffin using the alcohol–n–butyl alcohol schedule of Randolph (8). All sections were cut at a thickness of 12 microns using a rotary microtome. The paraffin was dissolved in xylol and the sections dehydrated using ethyl alcohol. The sections were stained using a modification of Johansen's quadruple stain (7). The slides were made permanent by mounting in Canada balsam.

RESULTS AND DISCUSSION

Cytological Effects of CDAA

The effect of CDAA on the number of dividing cells in longitudinal sections of barley and pea roots is presented in Table 1. The

Table 1. Effect of CDAA on the number of dividing cells in meristematic region. *

Concentration	Number of dividing cells	
	Barley roots	Pea roots
Control	33.7	76.3
1 ppm	30.4	73.2
10 ppm	8.2	68.7
100 ppm	1.5	62.4

*Average of 100 longitudinal sections, 12 microns in thickness.

barley roots grown in 1 ppm CDAA showed only a slight decrease in number of dividing cells, indicating a subtoxic dosage. Those grown in 10 ppm had relatively few dividing cells and those treated with 100 ppm had practically none. A slight disarrangement of cells was observed following treatment at the highest concentration.

The effect of CDAA on pea roots was less pronounced, indicating that peas are a comparatively resistant species. Although there was a progressive decrease in the number of dividing cells with increase in concentration, even at the highest dosage cell division in pea root tips was not seriously affected.

Cytological Effects of IPC

IPC at the lowest concentration studied (Table 2) completely inhibited cells division in barley roots. Root and cell structure

Table 2. Effect of IPC on the number of normal dividing cells in meristematic region*.

Concentration	Number of normal dividing cells	
	Barley roots	Pea roots
Control	33.7	76.3
1 ppm	0.	70.1
10 ppm	abnormal	56.9
100 ppm	abnormal	41.2

*Average of 100 longitudinal sections, 12 microns in thickness.

appeared normal but no dividing cells could be seen. When higher concentrations of IPC were used (10 and 100 ppm) cell structure and root structure were abnormal. Cells in the constricted tips were elongated and narrow, while cells in the swollen region were large and vacuolated. The orderly arrangement of cells into rows which was apparent in normal barley roots was not apparent in treated roots. Endopolyploidy was common and many large cells with several times the normal complement of chromosomes were observed (Figure 1). The chromosomes were scattered and appeared to be in a state of prolonged pseudo-metaphase. In some of these polyploid cells clumping of chromosomes could be observed with many of the clumps unequal in size or stuck together in long chains. This type

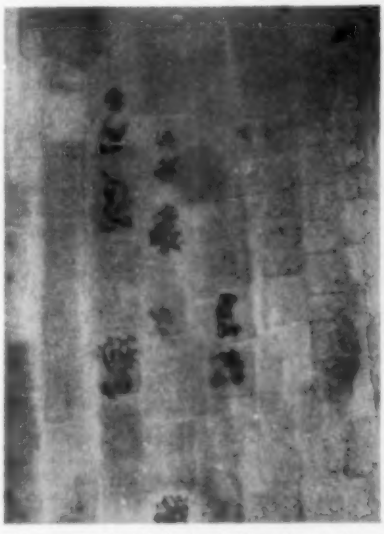

Figure 1. (Left) Polyploid cells in barley root tips resulting from treatment with 10 ppm IPC (× 390).

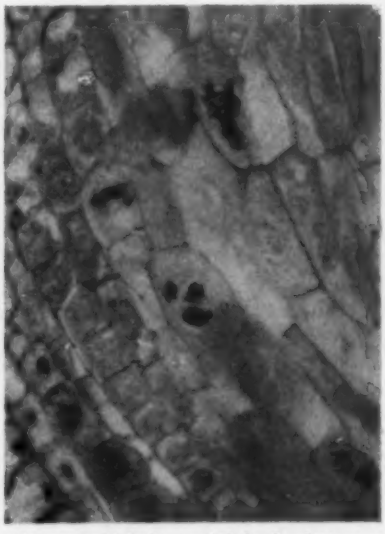

Figure 2. Triple nuclei in barley root tips treated with 10 ppm IPC (× 390).

of polyploidy could only result from repeated division of the chromosomes with no separation taking place. Further evidence to show that the spindle mechanism was affected was the presence of tripolar and polypolar anaphases. With the subsequent failure of the cell walls to form, these anaphases resulted in cells containing three or more restitution nuclei (Figure 2). These nuclei were often misshapen and unequal in size. Even when a bipolar anaphase separation occurred the cell wall often failed to form and a cell containing two nuclei resulted. Only in a very few cells did division appear normal.

Pea roots treated with IPC did not differ markedly from normal roots. However, as the concentration of chemical was increased there was a decrease in the number of dividing cells. Thus IPC appears to have only one effect on this resistant species, i.e. an inhibition of cell division directly proportional to concentration.

SUMMARY

Root tips of barley and peas which had germinated in concentrations of 1, 10 or 100 ppm solutions of CDAA or IPC, were collected 96 hours after the start of germination and prepared for cytological examination. CDAA inhibited cell division and subsequent root elongation in barley roots and the amount of inhibition was directly proportional to the concentrations of CDAA.

IPC at 1 ppm completely inhibited cell division in barley. At the two higher concentrations it caused endopolyploidy, binucleate cells and other abnormalities. Pea roots were only slightly affected in that fewer dividing cells were noted in treated roots as compared to untreated roots.

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The Decomposition of Herbicides in Soils[1]

PETER BURSCHEL[2] and V. H. FREED[3]

THE present extensive use of herbicides in soil application has resulted in a great deal of interest in the breakdown of these chemicals in soil[4] (1, 4, 6, 8). An increasing demand exists for information that will permit fairly accurate predictions of the residual life of these chemicals[4] (4). The breakdown of chemicals in soil has been repeatedly shown to be the result of microbiological activity in part but other factors are also known to contribute to the disappearance (1, 4, 5, 7, 8, 9).

The disappearance of herbicides from soil is a very complex phenomenon. It involves not only the nature of the compound, its adsorption by the soil, but also the properties of the soil, the amount of rainfall and leaching velocity, the temperature, as well as the amount of chemical added to the soil[4] (7, 8). In order to explore the process systematically, experiments must be designed that permit separation of the variables. Of the several pathways by which the chemical is lost from the soil, detoxification by microorganisms is one of the more important. The microbiological breakdown of 2,4–dichlorophenoxyacetic acid was shown by Audus (1) after an initial lag period to follow a curve resembling that of a reaction following first order kinetics. He subsequently identified at least one of the organisms responsible for the attack of the chemical. In certain instances this attack is important in the activation of the compound, as is found with 2,4–dichlorophenoxyethyl sulfate (9). Vlitos (9) demonstrated that it was necessary for this compound to be attacked by soil microorganisms which converted it at a slow rate into 2,4–dichlorophenoxyacetic acid, which was the compound primarily responsible for the activity against germinating broadleaved plants. Hill et al. (4) in the study of microbiological breakdown of 3–(*p*–chlorophenyl)–1,1–dimethylurea found that in contrast to the rate of breakdown of 2,4–D, a steady state of breakdown of the urea product was obtained which indicated that it was much less readily attacked by microorganisms and persisted for substantially longer times. Their data would suggest that the rate of breakdown may have been limited by the adsorption of the chemical by the soil. Sund (8) in his study on the behavior of 3–amino–1,2,4–triazole (amitrol) in soil presents data showing difference in rate of breakdown in different soil types. The data indicate that ultimate breakdown is due to microbiological attack.

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[1]Technical Paper No. 1079, Oregon Agricultural Experiment Station, Department of Agricultural Chemistry, Corvallis, Oregon.

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[3]Associate Chemist, Department of Agricultural Chemistry, Oregon State College.

[4]Burschel, P. Untersuchungen über die wirksamkeit von CMU und 3 Chloro IPC als herbicide in der forst wirtschaff. Thesis, University of Gottingen. 1955.

The purpose of this study was to investigate the rate of microbiological decomposition of three organic herbicides in the soil at two different temperatures. The object of following the rate at two different temperatures was to ascertain the kinetics of this phenomenon. From both first principles and observation that most microbiological processes follow first order kinetics, the deduction may be made that the decomposition of herbicides in the soil should likewise follow first order kinetics. If systematization of the information on the detoxification of herbicides in soils is possible, then one should be able to apply the Arrhenius (3) equation and determine the so-called "heat of activation." This could provide a basis from which it would be possible to make fairly accurate predictions as to the length of time that a chemical would be active in the soil at various temperature conditions, assuming that there was adequate soil moisture for microbiological activity.

MATERIALS AND METHODS

Chehalis loam soil, air dried to 1.5% moisture, was screened through a 20 mesh screen. The soil was then placed in gallon glass jars and the chemical added. IPC and CIPC used in the study had first been dissolved in acetone while the amitrol was applied as an aqueous solution. After evaporation of the solvent, the soil was thoroughly mixed for 1 hour on a tumbler mixer to assure uniform distribution of the chemical in the soil. Each treatment was replicated. The chemicals used in this study were recrystallized IPC (isopropyl N–phenylcarbamate) and CIPC (isopropyl N–(3–chlorophenyl)carbamate) and amitrol. The IPC was added to the soil at the rate of 2 and 4 ppm, the CIPC at the rate of 4 and 8 ppm, and the amtitrol at 20 and 40 ppm. The level of concentration for each of the chemicals was chosen on the basis of preliminary experiments which had determined that the concentrations used were those which were required to give a substantial effect in the first periods of the experiment but yet would break down sufficiently rapidly to permit a measurement of the rate.

After thorough mixing of the soil, water was added to bring the moisture content of the soil to 50% of its field capacity. The jars were closed and held at 29° C and 15° C. Thereafter, triplicate 75 gram samples were removed periodically and used for assay of the amount of chemical remaining in the soil. The periods used for the various chemicals were 7 days for the CIPC and amitrol and 2 days for the IPC. The samples were placed in small containers and 10 seeds of grey winter oats were planted in each. A control planting was grown at each time of planting. Prior to the experiment, dosage response curves had been obtained on 8 different concentrations of each chemical replicated three times. From the bioassay curve, it was possible to estimate the amount of chemical remaining in the soil at each planting date after converting the growth into percentage of the control. For amitrol, the disappearance of the chemical was also followed by the analytical procedure as outlined by Sund (8). Following planting, the containers were moved to a greenhouse at 65° F

and grown for 2 weeks. Growth height was then measured and the plants harvested and weighed.

The growth in each treatment, after calculation, was transformed into concentration of chemical. These figures were then used to calculate the amount of chemical remaining in the soil. Following the statistical analysis of the data, the regression line calculated by the least squares method was drawn for each compound at each dosage level and temperature. Using the values obtained from this line, the reaction rate constant was calculated and from this the heat of activation. Half-life of the chemical was also determined. The soil used throughout this work was a sandy loam having a pH of 6.9 and containing 2.8% organic matter, 36% silt and 24% clay.

RESULTS AND DISCUSSION

The data obtained in this study on amitrol are presented in Table 1. Each figure presented in the table is an average of three replicates. Inspection of Table 1 immediately shows a variation in rate of break-

Table 1. ATA remaining in soil at different times when stored at 15° and 29° C.

Temp. °C	Original conc. ppm	Percent of original conc. remaining, weeks after treatment						
		1	2	3	4	5	6	7
15	20	95.0	90.0	86.0	66.5	73.5	48.0	63.0
29	20	87.5	80.0	63.5	47.0	45.0	30.0	—
15	40	88.3	80.2	86.3	69.3	67.8	53.3	66.3
29	40	84.3	69.5	62.3	55.8	49.0	43.0	43.2

down with temperature. However, it is interesting to note that within reasonable limits the half-life of the compound at the two rates at a given temperature is nearly the same. This would tend to lend further support to the observation that this process takes place at a first order rate.

Table 2 presents the information obtained on CIPC and again each figure represents an average of 3 values. It will be noted in this table, in contrast to Table 1 on amitrol, that although the concentration is very much less, the rate of breakdown is quite slow. On the basis of these data the heat of activation for the breakdown of CIPC would be predicted to be much greater, perhaps in the order of three times that of amitrol. This observation is further substantiated if the comparison of the amount of the different chemicals broken down per gram of soil per day reduced to the basis of an original

Table 2. CIPC remaining in soil at different times when stored at 15° and 29° C.

Temp. °C	Original conc. ppm	Percent of original conc. remaining, weeks after treatment						
		1	2	3	4	5	6	7
15	4	100.0	100.0	100.0	92.5	82.5	75.0	68.8
29	4	95.0	82.5	55.0	37.5	32.5	0.0	0.0
15	8	100.0	97.5	95.4	90.8	85.0	81.9	79.4
29	8	100.0	85.0	71.3	70.0	69.8	48.3	15.0

concentration of 1 ppm is made. Taking the highest concentrations at 29° C, one finds that CIPC breaks down at the rate of .0106 micrograms per gram of soil per day, whereas the amitrol breaks down at the rate of 0.33 micrograms per gram of soil per day, or more than three times as fast.

The results obtained with IPC are given in Table 3, and again the figures represent an average of three values. Again attention is focused on the similarity of half-life of the two different concentrations at comparable temperatures. Here, too, the half-lives are sufficiently similar to support the conclusion that the decomposition of the herbicides in the soil follows a first order reaction.

Table 3. IPC remaining in soil at different times when stored at 15° and 29° C.

Temp. °C	Original conc. ppm	Percent of original conc. remaining, days after treatment						
		4	8	12	16	20	24	28
15	2	n	61.0	39.0	26.5	0.0	0.0	0.0
29	2	57.7	29.0	4.5	0.0	0.0	0.0	0.0
15	4	n	n	46.5	44.3	39.3	31.3	22.5
29	4	n	42.5	24.3	8.8	5.5	0.0	0.0

The data presented in Table 4 are the first order rate constants in terms of reciprocal time and the heat of activation for the compounds. The values presented for the rate constants are an average of three or more separate determinations. In general, these values

Table 4. The rate constants and heat of activation of the decomposition of three herbicides.

Compound	Temperature °C	k (day^{-1})	ΔH_a
ATA	15	2.25×10^{-2}	
	29	1.47×10^{-2}	5374
IPC	15	4.67×10^{-2}	
	29	8.79×10^{-2}	7768
CIPC	15	4.25×10^{-3}	
	29	2.39×10^{-2}	21,247

did not vary more than 15 percent, giving a quite remarkable uniformity of the rate constant. The uniformity of the value for the constant is further substantiation of the validity of the assumption that the decomposition is essentially first order.

Of great interest is the fact that the findings of the length of residual life of the chemical in this study parallel findings made in other trials (2) and in field observation. These data indicate that the order of increasing residual life of the chemical in the soil at equal concentration is amitrol, IPC, and CIPC. The actual rate of breakdown found at the highest concentration at 29° C was 1.31 micrograms per gram of soil per day for amitrol, .25 micrograms per gram of soil per day for IPC, and .085 micrograms per gram of soil per day for CIPC. This is parallel to the findings of the magnitude of the heat of activation required for the breakdown of these chemicals, as indicated in Table 4.

On the basis of the foregoing data, it would seem possible to make fairly accurate predictions as to the length of residual life of herbicides in the soil on the basis of the heat of activation. In making this prediction it is assumed that there is adequate soil moisture for microbiological activity. Recognition must be taken of the fact that such things as leaching and autodecomposition of the compound may accelerate the loss, thus introducing an error. The solution of this problem for the various herbicides would probably give a more reasonable basis for predicting the soil life of organic herbicides.

As techniques for this type of study are improved, it will be of importance to investigate the rates of the various types of decomposition separately. Such investigations should permit quantitative evaluation of each process.

SUMMARY AND CONCLUSIONS

The foregoing presentation has been concerned with the measurement of the rate of breakdown of IPC, CIPC and amitrol in the soil at two different temperatures. The data presented appear to support the conclusion that the decomposition process behaves as a first order reaction. It is possible on this basis to apply the Arrhenius equation to calculate the heat of activation required for this breakdown. Such calculations have been made and values presented for the three herbicides studied.

A consequence of these findings is that it may be possible to utilize such information in estimating the length of residual life of herbicides under field conditions. Additional work is needed to ascertain whether there is variability of the heat of activation with moisture level and soil type. However, such data are readily obtained in this type of study and would provide a reasonable basis to guide weed workers in estimating the length of residual life of the various herbicides in the soil under varying conditions.

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Control of Johnsongrass (*Sorghum halepense* L. Pers.) in Ohio by Herbicides and Cultural Practices[1]

E. O. Burt and C. J. Willard[2]

On rich alluvial soils where corn is the principal crop, Johnsongrass is the most serious perennial weed in Ohio at least as far north as the 40th parallel, and it has spread rapidly north of that area, even into southeastern Michigan. Many people still credit the statements of Piper (3), Vinall (6), and Bennett (1) that Johnsongrass behaves as an annual north of the 38th parallel. Pammel and King (2), to be sure, reported Johnsongrass as a perennial weed in southwestern Iowa in 1919 and the junior author's experience with it as a weed in Ohio, from the 40th parellel south, began in 1918. At Columbus these infestations have lived through winters in which the temperature was below 0° F for prolonged periods and the ground was frozen to a depth of 6 to 8 inches. There seems to be no hope for the eradication of Johnsongrass by mere freezing.

The experiments reported herein were field studies to evaluate certain herbicides and cultural practices when used alone and in different combinations, at several stages of growth, for control of Johnsongrass. The experiments were conducted from 1951 to 1954 on natural, uniformly severe infestations of Johnsongrass located on the flood plain of the Scioto River in southern Ohio.

Methods and Materials

Six experiments were conducted in which either split-plot or randomized block designs with three or four replications were used. The cultural and herbicidal treatments which were used in Experiments I through IV are listed in Tables 1 and 2. Rates of TCA refer to pounds acid equivalent per acre. Rates of sodium chlorate and CBM[3] (Polybor-chlorate) refer to pounds of commercial material per acre. Herbicides were applied as sprays using a "bicycle" sprayer of the type described by Shaw (5). The amount of regrowth of Johnsongrass was estimated in the next growing season following treatment. The data are estimated percent kill of Johnsongrass plants from rhizomes in the treated plots compared to the untreated checks. Yields of corn were also obtained the season following treatment. The ears from two corn rows 2 rods in length were husked and weighed. The percentages of moisture in the grain were determined and yields converted to 15.5 percent moisture.

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[1]In large part from a dissertation submitted by the senior author in partial fulfillment of the requirements for the degree Doctor of Philosophy at The Ohio State University.

[2]Assistant Agronomist, Florida Agricultural Experiment Station, Gainesville, Florida; and Professor of Agronomy, The Ohio State University, Columbus 10, Ohio, respectively.

[3]Sodium chlorate—25%, sodium pentaborate—58%, and sodium tetraborate—15%.

Experiment I. In the first experiment, main plots, 14 by 40 feet, consisted of chemical treatments. Plowed and unplowed strips, which extended across all of the replications, divided each of the main plots into two sub-plots, 7 by 40 feet. The plowed strips were plowed and disked once on September 12, 1951. Herbicides were applied September 14, 1951.

Experiment II. The same design was used in the second experiment. Main plots, however, were 28 by 21 feet, and sub-plots 14 by 21 feet. The plowed strips were plowed and disked once on October 23, 1951. Herbicides were applied the same day.

Experiment III. The design of the third experiment was the same as the first except that sub-plots were 14 and main plots 28 feet wide. The plowed strips were plowed and disked once and herbicides were applied on November 20, 1951. Experiments I, II, and III were conducted on land which produced corn in 1951. Each included 3 replications.

Experiment IV. This experiment was conducted on land used for beef cattle pasture in 1952. A split-split-plot design was used. Main plots were 266⅔ feet by 50 feet; main plot treatments consisted of four dates of plowing, July 30, August 20, September 10, 1952, and March 21, 1953. The strip plowed July 30 was disked once on August 25; the others were not tilled until the next spring in preparation for corn planting. Main plots were not randomized within the experiment. Sub-plots, 133⅓ feet by 50 feet, consisted of two dates of applying chemicals, September 15, 1952, and October 15, 1952. Sub-subplots, 26⅔ feet by 50 feet, consisted of five treatments: no herbicide, sodium TCA at 20 and 40 pounds per acre, and sodium chlorate at 100 and 200 pounds per acre.

Experiments V and VI. Sodium TCA at rates of 0, 10, 20, and 40 pounds per acre was applied to triplicate plots, 14 feet by 42 feet, of Johnsongrass sod on March 18, 1952. In 1953, TCA was applied at the same rates to quadruplicate plots, 13⅓ feet by 50 feet, on one of three dates; March 7, March 21, or April 4.

RESULTS AND DISCUSSION

Autumn herbicidal treatments. The CBM at rates of 100, 200, and 400 pounds per acre was ineffective in controlling Johnsongrass (Table 1) and was seriously toxic to corn the year following treatment. The stand of corn was reduced by about 30, 65, and 90 percent, respectively, in plots treated at the three rates. The corn that did survive was severely stunted throughout the growing season. By July 15 of the year following treatment, Johnsongrass was more vigorous in plots treated with the CBM than in the untreated plots, presumably because of the reduced competition from other weeds and corn. Although flowering was delayed about 10 days, Johnsongrass produced seeds which germinated and grew normally when planted the following spring.

In 1951 (Table 1), TCA at the rate of 60 pounds per acre gave the best control (85 perçent). Sodium chlorate at 150 pounds per acre was almost as effective. In general, TCA at rates of 20, 40, and 60

Table 1. Experiments I, II and III. Estimated percent control of Johnsongrass from treatments applied in 1951, as estimated on June 14, 1952.

Treatment	Rate lb/A	Johnsongrass control, pct, treated					
		Sept. 14, 1951		Oct. 23, 1951		Nov. 20, 1951	
		Plowed	Not pl.	Plowed	Not pl.	Plowed	Not pl.
No herbicide	—	23	0	10	0	15	0
Disked twice	—	33	0	—	—	—	—
TCA	20	57	40	35	10	23	17
TCA	40	80	63	70	36	52	27
TCA	60	—	—	85	35	—	—
$NaClO_3$	50	—	—	23	10	—	—
$NaClO_3$	100	58	45	57	30	43	27
$NaClO_3$	150	—	—	80	33	—	—
CBM	100	—	—	5	0	23	0
CBM	200	—	—	3	0	—	—
CBM	400	—	—	5	10	—	—

pounds per acre gave results similar to 50, 100, and 150 pounds of sodium chlorate, respectively. Applications of TCA and sodium chlorate made in September were more effective than those made in October, and these, in turn, were more effective than those made in November.

Plowing before applying herbicides consistently gave better results than leaving the land unplowed (Table 1). Herbicides applied in 1951 to land that recently had been plowed gave an average of 61 percent control of Johnsongrass. The same treatments, applied at the same time to land that was not plowed prior to treatment, gave an average of 22 percent control.

Minimum doses for September applications of herbicides for commercial control of Johnsongrass were 40 pounds per acre of TCA or 100 pounds of sodium chlorate. Higher rates were necessary if the land was not plowed prior to treatment.

TCA in 1952 was relatively less effective than in the previous year (Table 2). Sodium chlorate gave generally better results in 1952 than in 1951. The variation in the results probably is related to differ-

Table 2. Experiment IV. Estimated percent control of Johnsongrass top growth from rhizomes, June 20, 1953, and yields of corn, 1953, as affected by cultural practices and herbicides applied in 1952.

Herbicide	Rate lb/A	Johnsongrass control, pct, land plowed					Corn yields, bu/A, land plowed				
		July 30	Aug. 20	Sept. 10	Mar. 21	Means	July 30	Aug. 20	Sept. 10	Mar. 21	Means
None	—	84	50	14	2	38	69	49	31	28	44
TCA	20	88	52	9	1	38	62	46	25	28	45
TCA	40	90	68	24	15	49	89	56	35	30	52
$NaClO_3$	100	95	85	66	35	70	64	54	38	41	49
$NaClO_3$	200	99	96	87	75	89	58	45	32	39	44
LSD 5%				11		5			10		5
LSD 1%				16		7			14		6
Means		91	70	40	26		72	50	34	33	
LSD 5%				5					6		
LSD 1%				9					10		

ences in rainfall following treatment. The season was very dry in 1952; rainfall from September through December totaled 6.8 inches, compared to 14.6 inches in 1951 for the same period. Peters[4] and others have shown that the effectiveness of TCA is greatly dependent upon the amount and distribution of rainfall. On these soils sodium chlorate is usually leached from the soil during the winter, but was not in the unusually dry winter of 1952–1953, resulting in injury to the 1953 corn crop.

There was a high positive correlation between Johnsongrass control and yield in the plots treated with TCA. Soil moisture was low at the time ears were forming and the lack of moisture in plots containing considerable Johnsongrass resulted in low yields of corn. In spite of the good control of Johnsongrass obtained, the yield of corn was low in plots treated with sodium chlorate because of the toxic effects of the residual chlorate. The yield of corn in these plots was reduced in direct proportion to the amount of chlorate applied.

Spring herbicidal treatments. Early spring application of TCA at rates of 10, 20, and 40 pounds per acre gave 54, 83, and 99 percent control, respectively, of Johnsongrass plants from rhizomes (Experiments V and VI). Results indicate that corn can be planted 60 days following applications of TCA at 20 pounds or less per acre. A somewhat longer waiting period should elapse after an application of 40 pounds per acre.

Cultural practices. Plowing in the summer or fall followed by sufficient tillage to keep the Johnsongrass regrowth less than 8 to 10 inches high, was effective in reducing the stand of Johnsongrass plants from rhizomes, which resulted in increased yields of corn compared with plots not plowed the previous summer or fall. Cultural practices were more effective the earlier in the season they were carried out. Plowing July 30, with no further treatment except one disking on August 25, resulted in 84 per cent control of Johnsongrass from rhizomes. Plowing 3 weeks later (August 20) reduced the stand of Johnsongrass only 50 percent, and plowing September 10 gave only 14 percent control. Plowing the following spring (March 21) gave practically no control of Johnsongrass from rhizomes.

Since in these experiments the earlier in the season the ground was plowed the better the control of Johnsongrass, a Johnsongrass control demonstration in cooperation with the Agricultural Extension Service was set up in 1953. Johnsongrass areas were plowed July 3, and July 20, 1953, and a check was left until March, 1954. Both summer-plowed areas were disked a total of three times with a 24-inch solid disk on August 12, August 19, and September 7. This disk turned under all of the top growth each time. The next year the area plowed July 3rd produced 126 bushels of corn per acre and it was clean enough of Johnsongrass so that a crop of corn could have been produced the next year as well.[5] That plowed July 20, although

[4]Peters, R. A. The use of trichloroacetates as herbicides for perennial grasses. Doctoral Dissertation, The Ohio State University, 1952.

[5]The authors are indebted to Gordon Ryder, Extension Agronomist, for obtaining these data and for extensive cooperation in the Johnsongrass studies.

disked the same number of times, made only 94 bushels per acre, and enough Johnsongrass went to seed in it so that it would have been impractical to produce corn the next year without some special treatment. The area plowed in March, 1954, produced 36 bushels per acre of nubbins and contained so much Johnsongrass that it was almost impossible to harvest with a corn-picker.

SUMMARY AND CONCLUSIONS

Johnsongrass is the most serious weed in rich river-bottom corn fields in Ohio south of the 40th parallel. It is spreading rapidly north of this area.

Cultural practices are sufficient to control Johnsongrass if one season can be devoted to them. On the rich high-value soils on which these experiments were conducted the most common cropping practice is continuous corn. This does not permit culture except between the corn rows until at least September and usually October. These experiments were planned to explore the possibility of using chemicals to control Johnsongrass in continuous corn.

Experiments with herbicides indicated that if Johnsongrass was plowed before September 15 (at least a month before frost) it could be controlled 70 to 95 percent by TCA applied at 40 to 60 pounds per acre or sodium chlorate applied at 100 to 200 pounds per acre, if also applied in September. The increases in yield of corn from these herbicidal applications often have not been sufficient to justify their use economically, and they are not recommended for general use in this way.

The later in the fall the herbicides were used the less effective they were.

Herbicides used without plowing were only one-half to two-thirds as effective as those used after plowing.

Plowing Johnsongrass under without using herbicides was increasingly effective as the plowing was done earlier in the season.

As a result of these experiments and later demonstrations it is now (4) recommended to plow Johnsongrass in Ohio when it is 18 to 36 inches high, but at least before July 1, and to keep the ground worked so that the Johnsongrass does not grow more than 8 to 12 inches high during the remainder of the season. Beginning early in the summer is the most important single step in this control.

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Some Interactions Between Nutrient Level (N, P, K, Ca) and Diuron in the Growth of Cotton and Italian Ryegrass[1]

S. W. Bingham and R. P. Upchurch[2]

The pre-emergence use of 3–(3,4–dichlorophenyl)–1,1–dimethyl-urea (diuron) offers a means of controlling weeds in cotton during the extremely critical period of stand establishment (1, 2, 6, 8, 9, 10, 11). The amount of diuron required to give effective weed control without injury to cotton has been observed to vary between locations. Factors such as temperature, moisture, light conditions, and physical and chemical properties of the soil may be involved in causing variation in the activity of this herbicide.

The effects of soil cations, anions, and pH on herbicides which ionize or readily undergo conversion in the soil are reasonably well known. Such is the case for chlorates, phenoxyacetates, and dinitrophenols (5, 6, 7). The effects on relatively non-polar, non-ionic herbicides which do not readily undergo conversion in the soil are less well known.

No studies have been found which relate the phosphorus and potassium content of the soil to the activity of the substituted urea herbicides. Studies (7, 12) have been reported which indicated that phosphate metabolism was upset by phenoxyacetate treatments resulting in an increase in the inorganic phosphorus content of the plants. When excess phosphorus was applied, the detrimental effects of the herbicides were reduced. Voigt (15) found that phosphorus uptake was increased by certain pesticides and decreased by others. Data in two reports (3, 4) indicated that major shifts occurred in the nitrogen metabolism of plants as a result of treatment with substituted urea herbicides, but the effect of nitrogen supply was not considered. Ries and Sweet (13) found no consistent effect of soil pH on the activity of monuron. The phytotoxicity of diuron as influenced by some of these factors was investigated in this study.

Methods and Materials

The study was conducted in four principal, successive greenhouse experiments from November 1954 to November 1955. In each of the four experiments one of the factors N, P, K, or pH was studied at four levels together with two soil types, two species, and four rates of diuron. A secondary experiment was conducted using higher rates of phosphorus than in the initial experiment. Each treatment in the nitrogen, pH, and first phosphorus experiment was replicated five

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times. The potassium and secondary phosphorus experiments contained only four replications.

All additives were thoroughly mixed with the soils under study. The compounds and rates used are indicated in Table 1. In each experiment the nutrients which were not under consideration were added to all treatments at the levels shown in bold face in Table 1.

Table 1. Rates and sources of nutrients used in four herbicide-nutrient experiments and a secondary phosphorus experiment.

Nutrient	Applied as	Rate, mg/100 g oven dry soil[a]
P	$Ca(H_2PO_4)_2.H_2O$	First exp. 0, 5, **10**, 20
		Secondary exp. 0, **10**, 20, 40, 80
K	KCl	0, **5**, 10, 20
N	$NaNO_3$	0, **3**, 6, 12
For pH[b]	$Ca(OH)_2$	Georgeville soil 0, **50**, 200, 400
		White Store soil 0, **50**, 125, 250

[a]Only one nutrient was studied in each experiment. In each experiment, the nutrients which were not under consideration were added to all treatments at the levels shown in boldface.
The weights of nutrients were calculated on the basis of P_2O_5, K_2O, N, and $Ca(OH)_2$.

[b]pH was determined by the 1:1 glass electrode method at planting and 3 and 6 weeks after planting. The average pH values obtained for the four pH levels were 4.6, 5.6, 7.0 and 8.1.

The rates of herbicide used in the first (phosphorus) experiment were 0, 2, 3, 4 ppmw (oven-dry soil weight) of diuron for cotton (*Gossypium hirsutum* L. Var. Coker 100 Wilt) and 0, ¼, ½ and ¾ ppmw for Italian ryegrass (*Lolium multiflorum* Lam.). For succeeding experiments the herbicide rates were reduced to 0, 1, 2, 3 ppmw of diuron for cotton and 0, ¼, ⅜, and ½ ppmw for Italian ryegrass. In each experiment, the herbicide supply was a 20 g per liter stock solution prepared at the beginning of the study and kept at room temperature.[3] The designated amounts of herbicide were mixed thoroughly with the soil for each container by hand kneading in enamel mixing basins.

The plants were grown in 1-quart containers without drainage holes. Six cotton seeds or 35 ryegrass seeds were planted in each container at depths of 1 inch and ¼ inch, respectively. The cotton plants were thinned to three plants per container at 2 weeks, two at 3 weeks and a single plant at 4 weeks after planting. The healthiest plant in each container was left for a final 2 weeks before harvesting. The ryegrass was not thinned. The soil moisture was kept at an optimum level for plant growth as in earlier studies of this type (14). All plants were watered the day before harvesting. Green and dry weights were recorded.

The chemical and physical properties of the two soils used are shown in Table 2.

RESULTS

The interaction of diuron and phosphorus in the growth of cotton (Figures 1 and 2). An addition of 20 mg of P_2O_5 per 100 g of White Store soil caused the green shoot weight of cotton to increase by 125

[3]Since diuron was not as phytotoxic in the later experiments as in the earlier experiments, the stability of diuron solutions stored under these conditions is questioned.

Table 2. Chemical and physical properties of soils used in nutrient-herbicide experiments.

Soil property	Georgeville silty clay loam	White Store sandy loam
Percent organic matter	1.8	1.8
pH[a]	4.6	4.7
Exchangeable cations[b] (me/100 g)		
Ca	0.70	1.00
Mg	0.55	0.60
K	0.16	0.11
Cation exchange capacity[c] (me/100 g)	6.10	4.35
Soluble phosphorus (ppmw)[d]	8	6
Mechanical analysis:[e]		
Percent clay (<0.002 mm)	22.6	6.6
Percent silt (0.05–0.002 mm)	66.3	27.1
Percent sand (2.0–0.05 mm)	11.1	66.3

[a]pH was determined by 1:1 glass electrode method.
[b]Calcium and potassium were determined with the flame photometer. Colorimetric method was used for magnesium.
[c]Cation exchange capacity was determined by leaching the soil with neutral ammonium acetate and direct distillation method.
[d]Phosphorus was extracted with dilute acid solution and determined in solution by molybdate vanadate method.
[e]Mechanical analyses were made by the hydrometer method.

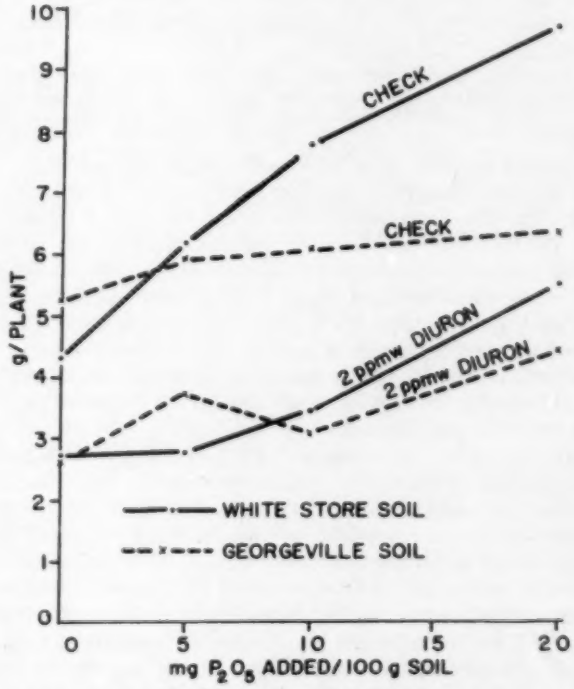

Figure 1. The influence of applied phosphorus on the response of cotton green shoot weight to diuron (first phosphorus experiment).

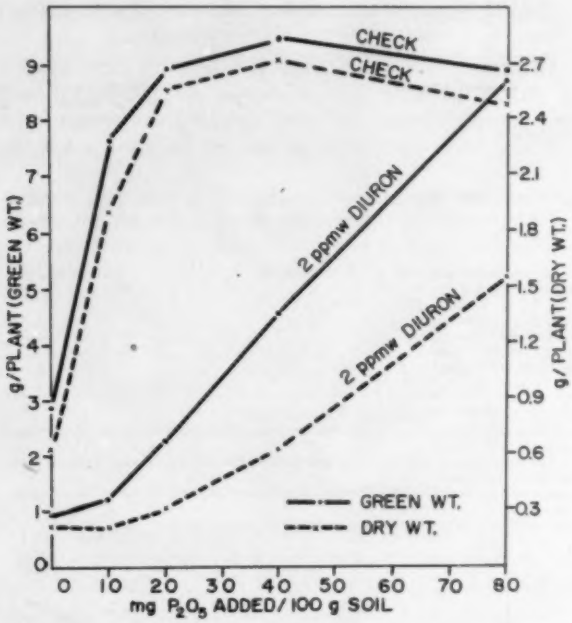
CHECK
CHECK
2 ppmw DIURON
2 ppmw DIURON
GREEN WT.
DRY WT.
g/PLANT (GREEN WT.)
g/PLANT (DRY WT.)
mg P_2O_5 ADDED/100 g SOIL
0 10 20 30 40 50 60 70 80
0 1 2 3 4 5 6 7 8 9
0.3 0.6 0.9 1.2 1.5 1.8 2.1 2.4 2.7

Figure 2. The influence of applied phosphorus (White Store soil) on the response of cotton green and dry shoot weight to diuron (secondary phosphorus experiment).

percent compared to cotton grown without the addition of phosphorus. The corresponding increase on Georgeville soil was only 22 percent. Under the best conditions used for plant growth, cotton grown on White Store soil had 50 percent more green shoot weight than cotton grown on Georgeville soil. Several rates of diuron were used, but only data from a rate having intermediate effects are presented in Figures 1 and 2 for illustrative purposes. The 2 ppmw rate of diuron used in the first phosphorus experiment produced a 37 percent reduction in green shoot yield of cotton where no phosphorus was added to White Store soil and a corresponding 50 percent reduction on Georgeville soil.

The addition of 5 and 10 mg of P_2O_5 per 100 g of White Store soil resulted in large increases in cotton growth in the absence of the herbicide but brought about only negligible increases in growth in the presence of an intermediate rate of diuron (2 ppmw). Increasing the P_2O_5 from 10 to 20 mg per 100 g of soil brought about increases in the growth of cotton in the presence of 2 ppmw of diuron. The increase in growth was similar to that obtained from lower increments of P_2O_5 in the absence of diuron. This interaction between diuron and phosphorus was significant at the 1 percent probability level.

Slightly different results were obtained on the Georgeville soil using the same rates of phosphorus and diuron as described above.

Although additions of phosphorus to Georgeville soil had little influence on the growth of cotton in the absence of herbicide, they tended to bring about slight increases in the green shoot weight in the presence of 2 ppmw of diuron. However, the interaction between diuron and phosphorus was not significant at the 5 percent probability level.

Dry shoot yields for the first phosphorus experiment, using White Store and Georgeville soils, showed trends similar to those obtained with green shoot yields. The only difference noted was that growth reductions due to diuron were less for green shoot yields than for dry shoot yields. This is attributed to the greater succulence of diuron-treated plants compared to untreated plants.

Since the above experiment showed interactions between phosphorus and diuron additions throughout the range of phosphorus rates used, a secondary experiment was conducted in which higher rates of phosphorus were used. Rates of 0, 10, 20, 40 and 80 mg of P_2O_5 per 100 g of soil were used with White Store soil. For the 0, 10 and 20 mg rates of P_2O_5 results similar to those for the corresponding rates on White Store soil in the first experiment were obtained (Figure 2).

Increasing the P_2O_5 rate to 40 and 80 mg brought no further increases in green or dry shoot yield in the absence of diuron but brought about large increases in green and dry shoot yield in the presence of 2 ppmw of diuron. The fact that 2 ppmw of diuron may reduce the green shoot yield of cotton by 84 percent or 3 percent depending upon the phosphorus status of the growth medium shows conclusively that phosphorus and diuron interact in some manner. This interaction was significant at the 1 percent probability level.

With one exception, diuron-treated plants were more succulent than untreated plants. Further, the addition of phosphorus tended to intensify this difference between treated and untreated plants.

The interaction of diuron and phosphorus in the growth of Italian ryegrass (Figure 3). In the absence of herbicide, the basic response of Italian ryegrass (green shoot yield) to additions of phosphorus to White Store and Georgeville soils was the same as for cotton with one exception. Somewhat greater increases in green shoot yield of Italian ryegrass than of cotton were produced on the Georgeville soil as a result of phosphorus additions. The ¼ ppmw rate of diuron used in Figure 3 for illustrative purposes produced 29 and 61 percent reductions on White Store and Georgeville soils, respectively, where no phosphorus was added. In the presence of diuron, the responses of ryegrass and cotton to phosphorus additions were similar in one respect but different in another. With cotton, any increment of phosphorus which brought about large increases in growth in the absence of diuron did not bring about large increases in growth in the presence of an intermediate rate of diuron. Conversely, the higher increments of phosphorus which failed to bring about additional increases in growth in the absence of herbicide brought about large increases in the presence of 2 ppmw of diuron. With Italian ryegrass, the first increment of phosphorus which produced

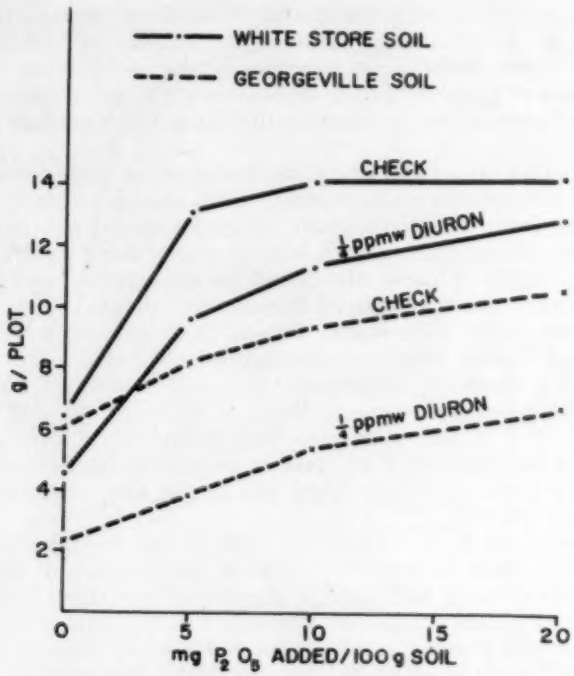

Figure 3. The influence of applied phosphorus on the response of Italian ryegrass green shoot weight to diuron.

large increases in growth of plants grown in the absence of herbicide likewise produced large increases in the presence of an intermediate rate of diuron. However, ryegrass was similar to cotton in its response to combinations of phosphorus and diuron in another respect. The final increments of phosphorus tended to produce greater increases in growth of plants grown in the presence of diuron than in its absence. For ryegrass, as in the case of cotton, the interaction between diuron and phosphorus was significant (1 percent probability level) for White Store soil but not significant (5 percent probability level) for Georgeville soil.

Dry shoot yields of ryegrass showed the same general trends as outlined here for green shoot yields.

The interaction of diuron and soil pH in the growth of cotton and Italian ryegrass (Table 3). Both cotton and ryegrass exhibited a well defined response pattern to variations in soil pH on both White Store and Georgeville soils. The best growth (green shoot weight) was most frequently obtained with a pH of 5.6 with lesser growth at pH 4.6 and 7.0 and the least growth of all at pH 8.1. These responses could have been due indirectly to pH since pH adjustments often induce changes in minor element availability. The data of Table 3 reveal that the responses of cotton and ryegrass were not the same

Table 3. The influence of diuron on the green shoot yield of cotton and Italian ryegrass when grown at various soil reactions on two soil types.

pH	Herbicide rate (ppmw)	Cotton (g/plant)		Herbicide rate (ppmw)	Grass (g/plot)	
		Georgeville soil	White Store soil		Georgeville soil	White Store soil
4.6	0	7.52	6.70	0	2.70	10.80
	2	6.84	5.56	¼	2.06	6.06
	3	3.80	4.30	⅜	.44	1.52
5.6	0	8.84	9.72	0	4.94	12.70
	2	7.30	9.32	¼	3.42	6.44
	3	5.70	5.16	⅜	.48	2.58
7.0	0	4.06	8.94	0	4.06	9.82
	2	4.14	7.74	¼	3.78	6.84
	3	2.86	4.18	⅜	.68	3.34
8.1	0	2.48	3.52	0	4.40	5.90
	2	2.40	3.04	¼	1.42	1.96
	3	2.08	2.12	⅜	.24	.12
LSD—5% level		1.45			2.69	
1% level		1.92			3.55	
CV—%		20			53	

to diuron at each of the levels of soil reaction studied. However, the relationships change so rapidly from pH level to pH level, from soil to soil, and species to species that a basic trend is difficult to establish from the data available. The one trend which seems to be best substantiated is that cotton plants grown in the presence or absence of diuron have the same pH optimum in these two soils whereas ryegrass plants grown in the presence of diuron tend to have a higher pH optimum than those grown in the absence of herbicide. Further studies of the interaction between soil reaction and diuron effects are needed. In such studies careful attention should be given to soil type and species of plant.

Table 4. The influence of diuron on the green shoot yield of cotton and Italian ryegrass when grown at various potassium levels on two soil types.

K_2O added to soil (mg/100 g)	Herbicide rate (ppmw)	Cotton (g/plant)		Herbicide rate (ppmw)	Grass (g/plot)	
		Georgeville soil	White Store soil		Georgeville soil	White Store soil
0	0	5.58	7.52	0	8.38	11.12
	1	7.00	7.22	¼	4.78	10.82
	2	1.70	2.22	⅜	.18	1.88
5	0	7.48	7.65	0	9.72	12.85
	1	8.38	7.20	¼	4.30	11.88
	2	1.95	1.22	⅜	.20	6.15
10	0	8.35	7.62	0	8.55	13.60
	1	7.12	8.45	¼	5.30	12.65
	2	1.02	1.38	⅜	.18	4.08
20	0	7.62	8.05	0	9.50	12.48
	1	8.65	7.30	¼	5.08	10.40
	2	1.85	4.15	⅜	.20	5.20
LSD—5% level		1.42			1.86	
1% level		1.88			2.47	
CV—%		18			19	

The interaction of diuron and potassium in the growth of cotton and Italian ryegrass (Table 4). Cotton and ryegrass responded little or not at all to additions of potassium to White Store and Georgeville soils. These two test species grown on White Store soil had a slight tendency to be less affected by an intermediate rate of diuron at the higher rate of potassium additions than at the lower rates. The effect of diuron on plants grown on Georgeville soil did not appear to be modified by additions of potassium. Additional experiments are needed in which potassium is studied at lower and more critical levels for plant growth.

The interaction of diuron and nitrogen in the growth of cotton and Italian ryegrass (Table 5). The addition of nitrogen to White Store and Georgeville soils did not increase the green shoot growth of the two test species used. The highest rate of nitrogen used even tended to have a deleterious effect. The addition of nitrogen did not greatly modify the response of cotton or ryegrass to an intermediate

Table 5. The influence of diuron on the green shoot yield of cotton and Italian ryegrass when grown at various nitrogen levels on two soil types.

N added to soil (mg/100 g)	Herbicide rate (ppmw)	Cotton (g/plant)		Herbicide rate (ppmw)	Grass (g/plot)	
		Georgeville soil	White Store soil		Georgeville soil	White Store soil
0	0	6.68	9.46	0	7.80	12.80
	2	5.98	6.80	¼	4.62	7.48
	3	4.00	4.70	⅜	1.04	4.32
3	0	6.98	9.16	0	8.00	12.20
	2	5.88	6.98	¼	4.76	9.50
	3	4.10	4.50	⅜	0.50	2.84
6	0	6.30	9.02	0	8.70	12.68
	2	4.84	6.50	¼	3.52	9.98
	3	3.52	4.26	⅜	0.90	4.62
12	0	6.16	8.48	0	6.72	11.62
	2	4.78	6.06	¼	4.80	9.52
	3	3.50	4.34	⅜	1.08	4.90
LSD—5% level		1.56			1.95	
1% level		1.98			2.57	
CV — %		19			24	

rate of diuron on either soil, but on both soils the toxicity of diuron to ryegrass tended to be less at the highest rate of nitrogen. The especially low yield of ryegrass on Georgeville soil at ¼ ppmw diuron and 6 mg rate of nitrogen, when compared to the untreated plants, is assumed to be anomalous.

DISCUSSION

The data presented in Table 6 on the chemical composition of cotton plants may provide an insight into the interaction which has been observed for diuron and phosphorus in the growth of plants. The plant analyses show that reductions in growth of the plants were not due to inhibition of phosphorus accumulation by the cotton shoots. On the other hand, the data show that diuron does interact

Table 6. The chemical composition of cotton plant shoots grown on White Store soil at three levels of phosphorus and three levels of diuron (dry weight basis).

P_2O_5 added to soil (mg/100 g)	Herbicide rate (ppmw)	Dry shoot weight (g/plant)	P_2O_5		MgO		CaO		K_2O	
			Percent	mg/plant	Percent	mg/plant	Percent	mg/plant	Percent	mg/plant
0	0	0.63	0.32	3.02	1.54	9.70	4.09	25.76	2.24	14.11
	1	0.44	0.49	2.16	1.94	8.54	4.41	19.40	2.83	12.45
	2	0.22	1.51	3.32	2.51	5.52	6.28	13.82	3.83	8.33
20	0	2.57	0.62	15.93	0.76	19.53	2.45	62.97	1.32	33.92
	1	2.47	0.59	14.57	0.80	19.76	2.22	54.83	1.30	32.11
	2	0.31	1.13	3.50	1.51	4.68	5.98	18.54	3.78	11.72
80	0	2.47	1.24	30.63	0.82	20.25	2.62	64.71	1.35	33.35
	1	2.66	1.24	32.98	0.82	21.81	2.77	73.68	1.32	35.11
	2	1.55	1.75	27.13	1.37	21.37	5.80	89.90	3.23	50.07
LSD—5% level			0.45	4.41	0.83	5.70	0.75	20.68	0.47	13.35
1% level			0.66	6.42	1.21	8.29	1.09	30.09	0.68	19.43
CV %			20	14	27	18	8	20	9	23

in some way with phosphorus. Greater additions of phosphorus were required for maximum growth of plants treated with the 2 ppmw rate of diuron than for untreated plants. Whether or not this interaction is related to the main mechanism whereby diuron controls plant growth is not known.

Even with the highest rate of phosphorus used, there was no indication that the maximum effect of phosphorus on diuron-treated plants had been obtained. Experiments to establish the maximum effect should provide useful data.

The effects of the phosphorus-diuron interaction on the cation status of plants also should be noted. The effect of diuron at 2 ppmw on the content of calcium and potassium in the presence of the highest level of phosphorus is noteworthy. Both the percentage and total weight per plant of these two elements were markedly increased by this treatment as compared to the high phosphorus check. The significance, if any, of this result is not apparent in the data of this study. Although the compound used to supply phosphorus also included a large quantity of calcium, this extra calcium was also available to the non-diuron-treated check.

Variation of levels of nitrogen, potassium, and pH in the soil had little effect on the growth response of plants treated with diuron. However, additional studies are needed in which these factors are studied at levels more critical for plant growth. The fact that cotton plants grown on diuron-treated soil in the presence of adequate phosphorus contained high levels of magnesium, calcium, and potassium would seem to minimize the possibility of diuron inhibiting the absorption and translocation of these elements. The possibility of effects through other modes of action is not eliminated by these data.

SUMMARY

The influence of levels of soil nitrogen, phosphorus, potassium, and pH on the response of cotton and Italian ryegrass to diuron herbicide on two soil types was investigated.

Phosphorus and diuron were found to interact to a large extent in affecting the growth of the two species. With cotton grown on White Store soil, small additions of phosphorus produced a large increase in the growth of plants grown in the absence of herbicide but produced little or no response for plants grown in the presence of an intermediate rate of diuron. Conversely, further addition of phosphorus caused very little increase in the growth of plants grown in the absence of herbicide but caused a large increase in the growth of plants in the presence of herbicide. Ryegrass, grown in the absence of diuron, responded much the same as cotton. In the presence of diuron, however, all additions of phosphorus acted to reduce diuron toxicity to the ryegrass.

On the basis of plant response and chemical composition of cotton plants grown in soil treated with various combinations of diuron and phosphorus, the postulation is advanced that phosphorus and diuron interact and that the effect of diuron on growth is partially regulated by the phosphorus level.

Soil nitrogen, potassium, and pH levels had little influence on the effect of diuron on the growth of plants in this study. However, nitrogen and potassium were not studied at levels which caused changes in plant growth in the absence of herbicide.

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Determining Minimum Amounts of Herbicide Needed for Aerial Brush Control[1]

EUGENE I. ROE[2]

SINCE the aerial brush control spraying operations in northern Minnesota have little research basis, the Lake States Forest Experiment Station has been carrying on some fundamental studies aimed at improving the results of this increasingly important aid to forest management. These consist of analyzing two factors basic to all foliage spraying jobs whether they are done from the air or from the ground. These are:

1. The minimum amount of herbicide (in terms of acid equivalent) required to control a unit-area of brush of a given species and density, and
2. The minimum amount of solution needed to distribute the herbicide uniformly over the sprayed area.

Tests involving the aerial application of known quantities of herbicide to known areas of brush are of little help in answering these questions. One can, of course, easily measure the amount of material leaving the plane, but determining the volume and distribution of the herbicide which actually falls on the test area is relatively difficult. This can be done only by sampling the material deposited on the brush by means of special cards or glass plates (2). However, to work out a reliable spray dispersion pattern many such samples must be taken in order to compensate for the variability in such factors as air speed, height of the plane above the brush, wind velocity and direction, temperature conditions, topographic irregularities and the like.

A more practicable approach is to determine the concentrations and volumes of herbicide needed for effective kill from applications which are much more exact than those made from an airplane flying 75 or more miles per hour, 25 to 75 feet above the brush. Once this basic information is worked out, the next job will be to devise aerial spraying techniques which will get the required amount of material on the foliage in a uniform manner.

A method being used to determine these two factors in northern Minnesota simulates aerial spraying by employing high-concentration, low-volume applications; the main difference is that the solutions are applied directly above the brush and are confined to prevent drift. The herbicide can be applied fairly rapidly and uniformly and the equipment used is simple, easily assembled, and not too expensive.

In general, the method consists of enclosing small, previously pre-

[1]Paper presented at 14th North Central Weed Control Conference, Des Moines, Iowa, Dec. 11, 1957.

[2]Forester, Lake States Forest Experiment Station. The Station is maintained by the Forest Service, U. S. Department of Agriculture, at St. Paul 1, Minn., in cooperation with the University of Minnesota.

Figure 1. Plot with enclosure in place but opened on sides to show typical brush. (U. S. Forest Service 482, 129)

pared plots of brush and then spraying them with herbicide materials at the desired concentration and volume (Figure 1). Four plots are sprayed at a time with each mixture.

THE ENCLOSURE

The enclosure, open at the top and the bottom, consists of a covering of 4-mil (0.004-in) polyethylene sheeting on a rigid frame made of galvanized thin-wall electrical conduit, fastened together at the corners by means of standard 2x4-in galvanized outlet boxes, equipped wth connectors and compression rings. The top and bottom sides of the frame form a square exactly 6.6 ft (inside dimension) on a side and thus enclose an area of 0.001 A; they are constructed of ½-in conduit. The four vertical sides consist of 5-ft lengths of ¾-in conduit; within each is a similar length of ½-in conduit. The latter have holes drilled at 6-in intervals into which a cotter pin can be set. This allows the top part of the frame to be raised and set at fixed levels ranging from 5 to 9½ ft. Thus the enclosure can accommodate brush up to about 9 ft in height.

The polyethylene sheeting consists of four panels, each 8 ft wide and 10 ft long. All edges are hemmed and the seams welded to prevent tearing.[3] Nylon ropes threaded through the top hems and tied to 1¼-in harness rings fastened to the top sides of the frame allow the panels to be drawn snugly to the top of the enclosure. After the enclosure is raised to the desired height, the panels are overlapped

[3]These panels were made to order by the Raven Industries, Sioux Falls, S. Dak.

at each corner and held tightly by means of furrier's clamps or 1¼-in bulldog paper clips.

SPRAYING EQUIPMENT

Since the tests being made on brush in Minnesota included volumes of emulsions ranging from 1 to 4 gal/A, special equipment was devised for the application of the small amounts (3.78 to 15.14 ml.) used per 0.001-A plot. This (Figure 2) incorporated suggestions

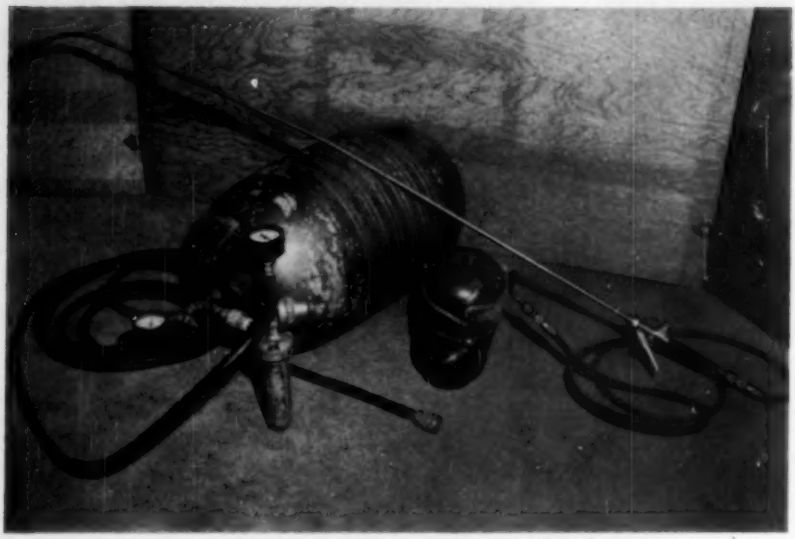

Figure 2. Equipment used in applying the herbicides. (U. S. Forest Service 484, 339)

from several sources (1, 3, 4, 5). The herbicides were applied from above by a man standing on a 10-ft. aluminum ladder with flat treads. Spraying was done at a pressure of 20 psi. with a single nozzle attached to a 4-ft. curved extension wand. The latter, which had a Spraying Systems Trigger Tee-Jet shutoff valve, was connected by a section of wire-reinforced neoprene hose to a 1½-qt. steel refrigerator tank.[4]

The latter, containing the herbicide emulsion, was connected to the air line by means of a speed coupler. Air was supplied from a tank of stainless steel with a capacity of 2100 cu. in. and a safety rating of 400 psi. This had a tire-type intake valve with pressure gauge, a shutoff valve, and a speed coupler connection with the air line, 20 ft. of ¼-in. heavy duty rubber hose. At the supply end of the latter was an air filter and a pressure regulator. Two tanks of air

[4]Safety rating 245 psi. To avoid contamination and to save the time and effort involved in line-cleaning between tests made with different herbicides, a separate system (coupler, reservoir, hose, shutoff valve, etc.) was used for each chemical.

filled in the morning to 150–160 pounds have proved sufficient for a day's spraying, the freeing of clogged nozzles, and line-cleaning operations.[5]

Herbicide emulsions were prepared in advance and taken to the field in 1-qt. plastic bottles. Since an application rate of 1 gal/A required only 3.78 ml of emulsion per 0.001–A plot, obviously such an amount would barely wet the inside of the line. Consequently, a sizable amount of the desired solution is added to the herbicide reservoir, and time used as a measure of the volume applied. The nozzles used are Spraying Systems flat spray No. 650017. These required 5 seconds to deliver water emulsions of 2,4–dichlorophenoxyacetic acid and 2–(2,4,5–trichlorophenoxy)propionic acid at the 1-gal/A rate on a 0.001–A plot. Although a longer period would be preferable for applying this volume, lower pressures proved impracticable with this particular nozzle, and no others were found which would give a lower output and at the same time be relatively free from clogging.

COST OF EQUIPMENT

Based on 1956 prices, the equipment described cost about $230. The most costly single item was the aluminum ladder at $85. The enclosure required an outlay of $75; of this, $45 went for the construction of the frame (electricians' rates) and $30 for the polyethylene panels. The airline (tank, hose, valves, reducer, etc.) came to about $37 and each separate herbicide system cost $30. The latter included expensive fittings and a sizable labor charge for assembly. Some reduction in cost could, of course, be made by using a lighter ladder and by constructing the frame of the enclosure with common labor.

CREW ORGANIZATION

Although it was intended that the enclosure would be stripped of its "curtains" every time it was moved to a new plot, the partial dismantling took considerable extra time and was soon stopped. A four-man crew has proven best, because of the heavy brush and low branches of trees which have to be avoided to prevent snagging the panels. In lighter, shorter brush or in a less comprehensive study (this one included 4 replications of 18 different treatments—2 herbicides, 3 concentrations, and 3 volumes—at each of 5 growth stages) where only short moves are involved, a two-man crew would probably prove satisfactory.

During the first season's operations, one man did the spraying from one side of the ladder, while the second man standing on the other side called time with a stop watch and uncovered and covered the nozzle at the beginning and end of the spray period. The third man turned on the air and watched the pressure, and the last man held the ladder which often could not be set up very firmly because

[5]An air blow gun will be found very useful in cleaning clogged nozzles. It can be attached to a short length of air hose with a female speed coupler unit on the other end so that it may be connected directly to the air tank.

Figure 3. Spraying in progress: man on right side of ladder operates the nozzle; one on left side keeps time and uncovers and covers the nozzle at the beginning and end of spray period; man at extreme left turns on air and checks pressure; fourth man steadies the ladder on rough ground. (U. S. Forest Service 482, 132)

of rough ground (Figure 3). In a smaller experiment on even ground, spraying, like moving the enclosure, could probably be handled effectively by two men.

EVALUATION OF RESULTS

Results were measured in terms of top kill and resprouting by height classes, based on a 100-percent stem count of each of the four plots in a given treatment.

Judging from the results of the first season's tests, coverage was generally satisfactory. The spraying equipment worked out very well. Clogging occasionally occurred even though a 200-mesh strainer was used in each nozzle, but this was troublesome only with a 50-percent solution of one of the herbicides used.

The enclosure also was satisfactory. The plastic sheeting proved flexible and surprisingly tough and tear-resistant. It was punctured rather frequently by branch stubs, but the holes usually did not spread. Major tears were few and were repaired on the spot with pressure-sensitive tape.

The frame proved very sturdy. Once erected, it was left as a unit until all 72 plots in one growth stage were sprayed; it required only an occasional tightening of the connectors and the locknuts. A 12-ft. welded frame made of ½- and ⅜-in. (inside diameter) aluminum tubing for use on tall mountain maple *(Acer spicatum)* on the Superior National Forest was much less satisfactory. Although this

enclosure was appreciably lighter, the frame was not sturdy enough and broke at several of the welds before the work was completed.

Where brush was not more than about 6 feet in height, the enclosure made of electrical conduit worked well with all common species, whether single-stemmed or clump-forming in habit. Even beyond this height, the single-stemmed species, such as hazel *(Corylus cornuta)* and chokecherry *(Prunus virginiana)*, could usually be confined with ease. On the other hand, tall stems of species such as mountain maple which have large spreading crowns could hardly be enclosed without breakage of some of the larger branches or serious damage to the plastic sheeting. The basic data on herbicide concentration and volume needed to control brush of this size and type can probably be obtained more easily by making enclosure tests on brush of the same species but of shorter height and adjusting the results accordingly.

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Mechanical Preparation of Pine Planting Sites in Florida Sandhills[1]

HAROLD E. GRELEN[2]

ALONG the lower Coastal Plain, from the western panhandle of Florida to the Carolinas, lie approximately ten million acres of deep sands. In northwest Florida the sandhills support chiefly scrub oaks and wiregrass *(Aristida stricta)*. Oaks are mainly turkey oak *(Quercus laevis)* and bluejack oak *(Q. incana)*. Once an understory to longleaf pine *(Pinus palustris)*, the scrub oaks gained dominance as heavy, indiscriminate logging removed the pines. Today only scattered pines remain, and the oaks and wiregrass prevent seed from these pines from restocking the area.

Sandhill soils have inherently poor moisture-holding capacity, and the water table is so deep that roots cannot penetrate to it. The shallow topsoil, sharply delineated by its darker color, goes down only 6 to 8 inches. In this shallow layer, feeder roots of all plants concentrate in a mat so dense that pine seedlings cannot successfully compete for moisture and nutrients (2).

In 1952, the Southern Forest Experiment Station, in cooperation with the Florida Board of Forestry, initiated research in northwest Florida to restore pine to the sandhills. Preliminary investigations proved that fire kills neither oaks nor wiregrass. Through the years, fire damage to oak tops has induced resprouting from root collars, resulting in the present stand of small trees.

Screening tests have so far failed to produce a satisfactory phytocide for eradicating the oaks. Wiregrass has resisted all chemicals, even heavy, uneconomical doses of several soil sterilants (3). Since wiregrass by itself can prevent satisfactory pine survival and growth, even a complete kill of oaks would not leave a suitable planting site (1).

An early study of mechanical methods of site preparation indicated that complete removal of all vegetation from planting sites increases pine seedling survival. However, growth differences—as between bulldozed and rootraked plots—emphasized the necessity for leaving as much as possible of the thin layer of sandhill topsoil. Good survival naturally must be followed by satisfactory growth.

In 1955, a new site-preparation study was installed to compare several machines and methods in current use by private companies. This paper reports the second-year results.

METHODS

Several private companies materially helped in establishing the study. Each company loaned machines and operators to apply its

[1]Presented at the joint meeting of the Weed Society of America and The Southern Weed Conference, Memphis, Tennessee, January 13, 1958.

[2]Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, Brewton, Alabama.

preferred treatment to study plots.[3] Treatments were installed with the following machines:

Marden brush chopper.—This tandem, double-drum chopper weighs 11 tons when drums are filled with water (Figure 1). Soil

Figure 1. Vegetative debris and topsoil remained on planting sites prepared by the 11-ton double-drum brush chopper.

compaction is naturally no problem in the sandhills. The two 7-foot drums rotate at counteracting angles oblique to the line of pull; this arrangement gives the blades a scraping action that uproots wiregrass and other perennial weeds. The chopper easily cuts up trees as large as 6 inches in diameter, and debris, as well as topsoil, remains on the site.

Rootrake-scalper.—An ordinary rootrake was converted to a "scalper" by welding a horizontal cutting blade to tips of the rake teeth (Figure 2). Operation and action of the machine are similar to that of a bulldozer. Oak removal is slow but thorough. Much of the topsoil is transported to the windrows along with the vegetation.

BSW Brush Cutter.—This big snowplow-like machine cuts a furrow approximately 8 feet wide (Figure 3). Depth of furrow is adjustable, but ranged between 6 and 12 inches in this study. The machine has a sawtooth cutting edge along the bottom of the blade, which severs oak stems as they are being side-swiped, along with topsoil, into windrows.

Plots were burned in May 1955, in an attempt to weaken the oaks and wiregrass. In June 1955 each of the three machines, pulled by heavy crawler-type tractors, treated a pair of 2-acre plots on each of three randomized blocks. In September 1955 one plot of each pair was retreated. The retreatment on the chopped plots was identical to the first treatment, the chopper moving in a circular direction.

[3]Companies and their machines were: chopper—Hardaway Contracting Company, Marianna Florida; rootrake-scalper—St. Joe Paper Company, Port St. Joe, Florida; BSW brush cutter—T. R. Miller Mill Company, Brewton, Alabama.

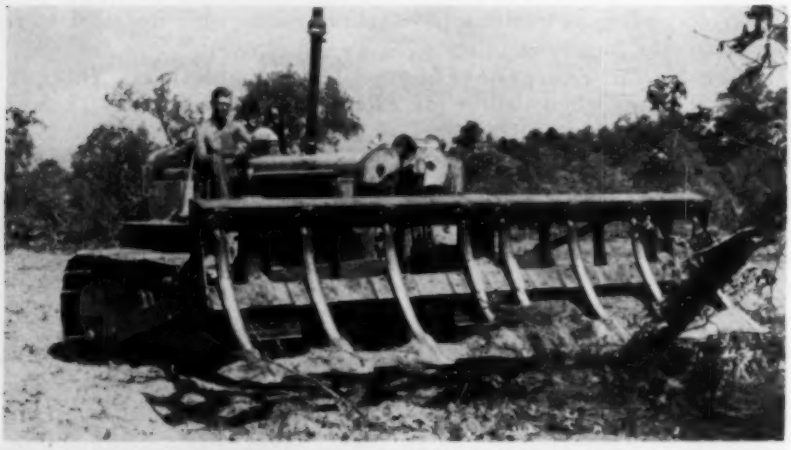

Figure 2. Considerable topsoil was removed by the rootrake-scalper as it pushed oak tops into windrows.

Furrows on the retreated BSW plots were spaced between those of the first treatment. Since a second treatment with the rootrake-scalper was not considered economical or practical, a heavy Rome disk harrow was substituted in the September retreatment.

Thus, three single and three double mechanical treatments, plus no-treatment and burn-only checks, were applied to three blocks of eight 2-acre plots—a total of eight treatments, replicated three times. One-year-old slash pine seedlings *(Pinus elliottii)* were planted at a 6- by 9-foot spacing on all plots in January 1956. A sub-plot enclosing

Figure 3. When in operation, the BSW brush cutter piles vegetation and topsoil in parallel windrows, leaving a planting furrow in practically sterile sand.

100 seedlings was established in the center of each 2-acre plot. Survival and height growth of the subplot seedlings are recorded annually.

RESULTS

At the end of two growing seasons, slash pine survival on the three double treatments averaged more than 80 percent (Table 1). Single treatments averaged 75 percent and checks, 60 percent. Best survival, 86 percent, occurred in the wide furrows of the double BSW treatment, but this was not significantly higher than the 83 percent average of the two next best survivals. A survival of 58 percent on the no-treatment check plots was significantly lower than that for any of the mechanical treatments.

Table 1. Average heights and survivals of slash pine seedlings at the end of two growing seasons.

Treatment	Height—ft	Survival—pct
Double treatments:		
Marden chopper	3.4	83
Rootrake-scalper	2.4	83
BSW	1.1	86
Single treatments:		
Marden chopper	2.3	78
Rootrake-scalper	1.3	75
BSW	1.4	73
Checks:		
No treatment	1.2	58
Burn only	1.2	63

Growth was best on the double-chopped plots, where trees averaged 3.4 feet in height at the end of their second growing season (Table 1, Figure 4). The next best average height, 2.4 feet, was on plots treated twice with the rootrake-scalper. Both treatments provided completely bare sites at planting time, with very little oak

Figure 4. Growth is best on the double-chopped plots, where planted slash pines averaged 3.4 feet in height after two growing seasons.

resprouting since. The difference in height growth between these two denuded sites is attributed to the removal of topsoil by the rootrake-scalper. The chopper removed no topsoil, though it disturbed the surface considerably. Greater uniformity of tree height and a healthier foliage color on double-chopped plots are ascribed to the more uniform layer of topsoil left by this treatment.

After 2 years, very few oaks and wiregrass plants were found on the severely denuded, double-treatment plots of the Marden chopper and rootrake-scalper. No wiregrass reinvasion has occurred, and the few surviving oaks have made little growth. Single treatments with the chopper and rootrake-scalper merely "cultivated" the oaks and wiregrass, and did not reduce competition enough to allow the pines a head start. At the end of 2 years, pines on the double-treatment plots of the chopper and rootrake-scalper were more than a foot taller than those on the single-treatment plots. Since there was less soil disturbance on the single treatments, height differences are attributed to different intensities of oak and wiregrass competition. Poor pine growth on the BSW plots was due to topsoil removal rather than plant competition.

CONCLUSIONS

At present, the best known method of preparing pine planting sites in the west Florida sandhills is clearing with a heavy, tandem double-drum brush chopper. This machine, with drums set at counteracting oblique angles to the line of pull, practically eliminates scrub oak and wiregrass competition while leaving the topsoil on the planting site. Vegetative debris also remains in place, contributing to the organic content of the soil.

Adequate survival of slash pine seedlings can usually be obtained by methods that provide a denuded planting site. If the topsoil is removed, however, satisfactory growth cannot be expected.

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The Comparative Toxicities of Monuron and Simazin in Soil[1]

T. J. Sheets[2]

In recent years the relationships of 3–(*p*–chlorophenyl)–1,1–dimethylurea (monuron) phytotoxicity and various factors in the soil have received considerable attention (1, 3, 5, 6). Similar information for 2–chloro–4,6–bis–(ethylamino)–*s*–triazine (simazin) is lacking because of its later development as a herbicide. The experiments reported here were initiated to compare (a) the toxicity of monuron and simazin in solution culture and in soil, (b) the inactivation of the two compounds in soil and, (c) their downward movement through soil columns.

Experimental Procedure

Oats (*Avena sativa*, L. Kanota) and soybeans (*Glycine max*, Merr. Chippewa) were employed as test plants in the solution culture test. Seeds of the two species were germinated in autoclaved sand. The oats were transferred to Hoagland's solution (2) when approximately 3 inches high. The soybeans were transferred when the hypocotyl and root were approximately 5 inches long and the plumule was about 0.5 inches long. The herbicides were added to the solutions the day following transfer at concentrations ranging from 0.01 to 0.32 ppmw for oats and from 0.02 to 0.64 ppmw for soybeans. Each culture consisted of 850 ml of treated solution contained in a quart Mason jar. There were 10 oat plants per culture and 3 soybean plants per culture. Fresh weights of tops of oats and fresh weights of growth above the cotyledonary node of soybeans were taken two weeks after transfer.

The initial and residual toxicities of the two herbicides were investigated in Yolo clay loam and in Hesperia sandy loam soils. Some properties of the soils are given in Table 1. The soils were

Table 1. Percent clay, percent organic matter, pH, and cation exchange capacity of the soils.

	Percent clay	Percent organic matter	pH	Cation exchange capacity[a]
Yolo clay loam	33.9	2.4	7.0	20.2
Hesperia sandy loam	16.0	0.3	7.5	9.8
Sacramento clay	79.5	4.8	5.6	—
Yolo sandy clay loam	22.5	1.4	6.8	—

[a]milliequivalents per 100 g of oven dry soil

[1]Cooperative investigations of the Crops Research Division, ARS, USDA, and the Calif. Agri. Exp. Sta. The experimental sample of monuron was supplied by E. I. DuPont de Nemours & Company, Wilmington 98, Delaware and that of simazin by Geigy Chemical Corporation, Yonkers, New York.

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screened, and 500 g oven dry portions were placed in No. 2 metal cans. The chemicals were added to the soils as water suspensions. The suspension for each culture was prepared by mixing the appropriate amount of chemical from a stock suspension with sufficient water to wet the soil to field capacity. Approximately uniform distribution in the soil was obtained by adding the suspension in three portions to three portions of the soil as the metal cans were filled. The concentrations were 0, 0.31, 0.62, 1.25, 2.5, 5.0, 10, and 20 ppmw of oven dry soil. In addition three lower concentrations (0.039, 0.078, and 0.156 ppmw) were used with the sandy loam soil. Following treatment, 13 oat seeds were planted per can. Approximately 30 days after seeding the fresh weights of tops were determined. The cultures were allowed to remain unwatered for the next 30 days. At the close of the second 30-day period the soil in each culture was pulverized and the plant residue from the previous harvest returned to the container. The cultures were wet to field capacity and seeded again. This system of cropping was continued for a total of five crops.

The initial toxicities of the two herbicides to soybeans were compared in Yolo sandy clay loam and in Sacramento clay. The cultures were treated as described above at concentrations of 0, 0.25, 0.5, 1.0, 2.0 and 4.0 ppmw of oven dry soil. Following treatment eight soybean seeds were planted per can; and after the seeds germinated, the cultures were thinned to two plants per can. Fresh weights of tops above the cotyledonary node were determined 30 days after seeding.

Upchurch (7) used GR_{50} (dsw) to indicate the amount of herbicide in ppm which reduced dry shoot weight 50 percent. In Table 2 and Figure 1 of this paper the results are presented as ED_{50} values (50 percent effective dose); i.e. the amount of herbicide in ppmw which reduced the fresh weight of the test plants 50 percent. For each concentration series within a replication an ED_{50} value was determined as described previously (6). Each ED_{50} was obtained by plotting the fresh weights as percentages of the control against the logarithm to the base 10 of the concentrations and constructing a free-hand curve to fit the points. The point on the concentration axis which corresponded with the point of intersection of the curve and the 50 percent yield level was the ED_{50} as a logarithm. The antilogarithm of the 50 percent point gave the ED_{50} in ppmw. The standard errors in Table 2 were computed from these values.

The movement of monuron and simazin in Hesperia sandy loam and in Yolo clay loam was studied. The herbicides were applied to the surface of Hesperia sandy loam columns at 1 and 20 lb/A. The surface of columns of Yolo clay loam were treated with sufficient monuron (6.5 lb/A) and simazin (3.0 lb/A) to reduce growth of oats 50 percent throughout the columns. The Yolo clay loam was also treated with the 20 lb/A rate of both herbicides. All herbicidal treatments received two rates of water (2 and 10 surface inches).

Soil columns 2 inches in diameter and 20 inches high were employed. They were constructed by attaching end to end with water resistant electrical tape six lidless and bottomless cans 2 inches in diameter and 2.875 inches high. An additional can of the same

diameter was attached to the top of the column; in this way space was provided for a water head. Still another can with the bottom intact but punctured (15 holes, each approximately 0.1 inches square) was attached to the lower end of the column. This can supported the soil column but allowed drainage. After the columns were filled with soil, they were wet to field capacity prior to treatment. The herbicides were applied to the soil surfaces as dry soil-chemical mixtures. Twenty four hours following treatment water was added as a water head. The columns were sectioned by removing the tape 24 hours after drainage from the bottom can ceased. Each section was placed, with its soil in place, in a petri dish; and 10 oat seeds were planted per section. Movement of the herbicides was determined by injury and fresh weights 3 weeks after seeding.

All tests were conducted in the greenhouse. Randomized block experimental designs were utilized. Except for the leaching study, which was duplicated, all experiments contained three replications.

RESULTS AND DISCUSSION

Injury symptoms of monuron have been described for several plants (4, 8). Symptoms of simazin injury to oats and soybeans growing in treated soil appeared very similar to those produced by monuron.

In solution simazin was more toxic to oats than monuron. Whereas 0.028 ppmw of simazin reduced the growth of oats 50 percent, 0.040 ppmw of monuron were required to cause the same degree of injury. The ED_{50} values for monuron and simazin on soybeans were not significantly different. In solution about 2.5 times as much simazin was required on soybeans as on oats; about 1.9 times as much monuron was required.

Results of the first cropping revealed that simazin was far more toxic to oats in both soils than monuron. In Hesperia sandy loam 2.5 times as much monuron was required to reduce the growth of oats 50 per cent. In Yolo clay loam simazin was 2.2 times as injurious as monuron. Both monuron and simazin were required in greater quantities in the clay loam soil. In Yolo sandy clay loam and in Sacramento clay simazin was more injurious to soybeans than monuron.

In these tests the initial toxicity of monuron was influenced more by the soil than that of simazin. The data in Table 2 show that in solution culture monuron and simazin were about equally injurious to soybeans. However in both Yolo sandy clay loam and Sacramento clay higher dosages of monuron than simazin were required to reduce the growth of soybeans 50 per cent. The same relationship existed with oats as the test plant. Although simazin was more toxic to oats in solution, even greater differences in the toxic level of the two herbicides occurred in the soil tests. These results might be interpreted to mean that simazin was not adsorbed as tenaciously as monuron.

The curves in Figure 1 depict the relationship of toxicity and time for the two herbicides in Yolo clay loam and Hesperia sandy loam.

Table 2. ED_{50} values of monuron and simazin in solution and in soils.

Medium	Oats		Soybeans	
	Monuron	Simazin	Monuron	Simazin
Solution culture	.040 ±.003[a]	.028 ±.001	.077 ±.006	.069 ±.002
Yolo clay loam	.93 ±.07	.43 ±.033	—	—
Hesperia sandy loam	.073 ±.005	.029 ±.003	—	—
Sacramento clay	—	—	2.14 ±.17	1.64 ±.09
Yolo sandy clay loam	—	—	.52 ±.13	.25 ±.01

[a]Mean ED_{50} value and standard error of the mean.

The 50 per cent effective dose, with oats as the test plant, was again used as a measure of the toxic level of the herbicides. These curves show that in both soils monuron phytotoxicity was lost more rapidly than simazin phytotoxicity. The rates of loss of active chemical can be determined from the slopes of the curves in Figure 1. For the

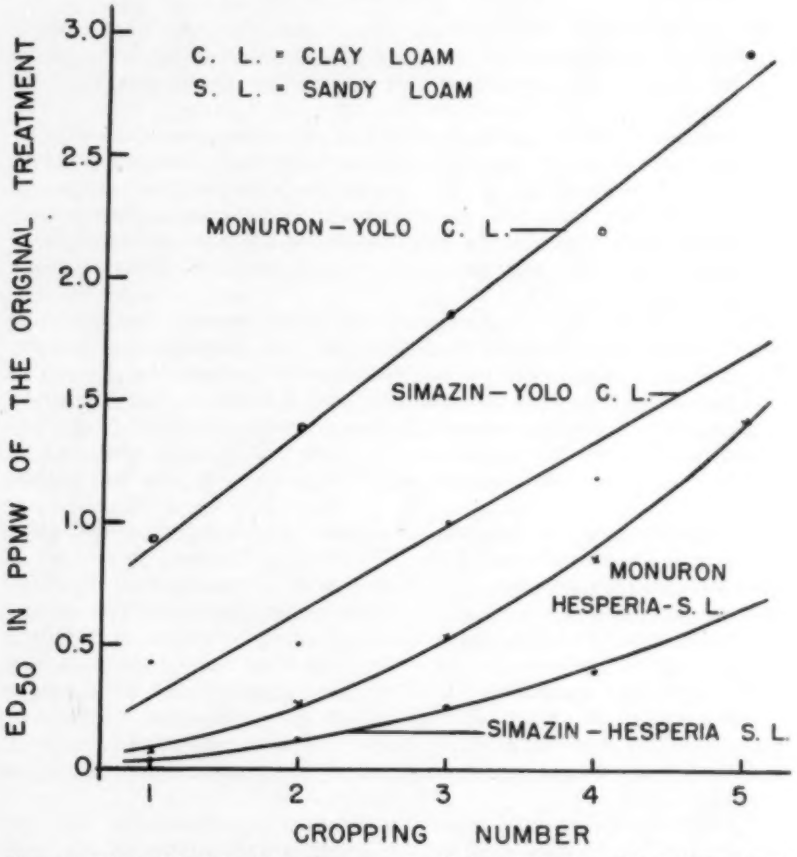

Figure 1. The residual toxicity of monuron and simazin in two soils as determined by the growth of oats above the soil level.

first three croppings (5 months) the inactivation rates of monuron and simazin were more rapid in the clay loam than in the sandy loam soil. Later the rates of toxicity loss of the two herbicides in the sandy loam soil approached the rates of loss in the clay loam. However at the end of the fifth cropping the toxic level of each herbicide in Hesperia sandy loam was lower than the toxic level of the respective herbicide in Yolo clay loam.

Monuron and simazin were displaced downward through soil columns by percolating water at about equal rates. In columns of Hesperia sandy loam, treated with 1 lb/A of herbicides and leached with 2 inches of water, symptoms on the oat plants indicated both chemicals were moved into the second section of the columns; but fresh weight data suggested that most of the applied herbicides remained in the top section of the columns. In columns treated with 1 lb/A and leached with 10 inches of water, definite symptoms developed in the lower four sections of monuron and simazin columns; no symptoms occurred in the top sections.

At the 20 lb/A rate in Hesperia sandy loam 2 inches of water caused movement of monuron and simazin into the top three sections in quantities sufficient to greatly reduce growth of the oat plants. There was slight movement of both chemicals into the fourth section. The oats were killed in all sections of columns of Hesperia sandy loam treated with 20 lb/A and leached with 10 inches of water.

Downward movement of monuron and simazin was much less through columns of Yolo clay loam when the same amounts of herbicides and water were applied to both soils. As in Hesperia sandy loam monuron and simazin were displaced at about the same rate with percolating water.

In the absence of leaching, monuron was inactivated more rapidly than simazin in the two soils. The herbicides were moved downward by water at approximately equal rates. Hence, on the basis of these tests, simazin might be somewhat more hazardous than monuron with respect to toxic soil residues and accumulation from repeated treatments. On the other hand the potentialities of simazin as a soil sterilant may be greater because of this property. Other factors such as soil type effects under field conditions, variations in plant susceptibility, and perhaps photo-decomposition must be investigated before a complete appraisal of the comparative efficiency of these two compounds can be made.

SUMMARY

The comparative phytotoxicities of monuron and simazin in solution, their initial and residual toxicities in soils, and their downward movement with percolating water through soil columns were investigated.

Simazin was more injurious than monuron in solution and in soils when oats were used as test plants. In solution monuron and simazin were about equally toxic to soybeans; but in two soils simazin was much more toxic. The data indicate that the initial toxicity of simazin was influenced less by the soil than that of monuron.

Under conditions of no leaching monuron was inactivated more rapidly in Hesperia sandy loam and in Yolo clay loam than simazin. The two herbicides were displaced downward through soil columns by percolating water at about equal rates. Downward movement of both compounds was greater through the sandy loam soil.

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Foliar Penetration of Herbicides—Review and Present Status[1]

H. B. Currier and C. D. Dybing[2]

Systemic herbicides applied to leaves must move from the leaf surface into uninjured phloem in sufficient quantity to be translocated throughout the plant. Contact herbicides must at least enter the leaf. Leaves of terrestrial plants are adapted to exchange of gases —water vapor, CO_2, O_2—but are not adapted to absorb foreign solutes in applied solutions and to translocate them systemically; insectivorous species and some water plants may be exceptions. Failure of herbicidal effectiveness often may be due to lack of penetration. However, selectivity towards herbicides is not clearly related to differential absorption (5, 82, 172, 176).

The typical mesophytic leaf may be but a fraction of a millimeter thick yet in translocation tests the time taken for 2,4–D to move from the cuticle into the phloem seemed to be greater than from leaf to root over distances of 100 mm. or more (39). Thus an organized transport system capable of rapid conduction of solute from cuticle to phloem apparently is non-existent. Diffusion is doubtless the principal mechanism; protoplasmic streaming may assist and enzymatic reactions may be involved, but proof is lacking.

The purposes of this paper are, first, to outline what is known about foliar penetration of herbicides, and second, to discuss existing problems requiring solution in this field of investigation.

The term penetration is used in a broad sense to denote passage of substances into the leaf and their entry into the various tissues, including vascular elements. Reference is made to the penetration of substances other than herbicides (e.g. nutrients) where the information contributes to understanding of the problems. Recent pertinent reviews are those of Crafts (25), Boynton (16), van Overbeek (164), Wittwer (177), Mitchell and Linder (112), and Woodford et al. (178).

Pathways of Initial Penetration

Regions of the epidermis. Both surfaces of the leaf function in absorption of chemicals. Usually the lower epidermis is more penetrable than the upper (12, 22, 58, 60, 68, 70, 117, 167, 175). This is probably due both to a thinner cuticle and more numerous stomata. However, species differ as to which surface functions most in absorption (69). Epidermis over veins is more penetrable to fluorochromes (149, 182), and, judging from subsequent translocation and response, to herbicides (29, 33, 96, 97, 164), and to other substances (62, 155, 168). Leaf-margin application is less effective than placement over the midrib (96, 168).

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Cuticular penetration. Entry of chemicals into the leaf is cuticular and sometimes stomatal. Intact, unperforated cuticle is a strong barrier to rapid penetration (119, 146, 164, 174), but there seems to be no question that herbicides and other substances penetrate it (12, 97, 142). It is a semi-polar layer, inert, not readily wetted by water, wetted by oils, but only slightly permeable to either solvent. Penetration of oils is evident in depressions just above the anticlinal walls (89). Cuticular areas over the anticlinal walls are preferentially penetrated by water-soluble substances (131). In young leaves cuticle may be absent over these areas (155). An outer covering of wax (bloom) of some cuticles (133) increases the lipid barrier to entry of polar molecules and decreases the adherence of aqueous spray droplets. Wettability is measured by contact angle (46) and is negatively correlated with amount of surface wax (100). Rubbing off the wax increases spray droplet retention (51), as well as permeability to water (146) and doubtless to all polar solutes. Cracks and perforations increase cuticular permeability (119). Thin and hydrated cuticles are more permeable to polar solutes than are thick and less hydrated ones. Sorption is the first phase in the cuticular penetratration process. Initially it is rapid, and is influenced by pH, polarity of solvent and solute, charge on the penetrating particles and additive particles, and concentration (119).

Fluorescent dye techniques (19, 42) clearly demonstrate cuticular penetration; the dye appears rapidly in the anticlinal walls of the epidermis. The same localization has been reported (58) for 3,5–dinitro–*o*–cresol (DNC). The transpiration stream moves in these walls (10, 18, 152).

No plasmodesmata have been demonstrated in cuticle, but similar structures exist in the cutinized outer epidermal wall (92, 135). These (ectodesmata) are reported to push into, and, alternately, to withdraw from pores in the wall (136). It is possible, but not yet demonstrated, that they may be important in foliar penetration. Chemical substances, e.g., histidine, stimulated the in and out movement of the protoplasmic strands, a process sensitive to concentration (136).

Electron microscopy confirmed the presence of pits and plasmodesmata in the outer epidermal walls of onion leaf, but no pores equal in size to the external wax rodlets were found (138). A pectinaceous layer, hydrolyzable by pectinases, separates the cuticle proper from the epidermal wall (118).

Alternate polar or apolar pathways across the cuticle have been suggested (126); water soluble herbicides may follow an aqueous path, and oil-soluble substances may follow a lipid path (28). Rouschal and Strugger (130) found small cuticular pores through which transpiration occurred preferentially.

Stomatal entry. There has been disagreement as to the penetration of aqueous spray solution through stomata. Most workers have claimed that it does not occur, at least not unless the surface tension is very low (27, 58, 116, 162, 164, 165, 170). Skoss (146) stressed the importance of stomatal entry of 2,4–dichlorophenoxyacetic acid (2,4–D).

Stomata have been claimed to be primary sites of entry for Co^{60} (68, 70), 1–naphthaleneacetic acid (NAA) (75), and H_2SO_4 (6). This path was not indicated for P^{32}, K^{42}, Rb^{86} (159), or urea (127, 167). On the basis of all available information one must conclude that both stomata and cuticle may be involved (22, 43, 164, 174)[3], with the stomatal component varying widely due to a complex of factors influencing stomatal opening. Stomatal entry of aqueous solutions appears especially dependent on an efficient surfactant at a suitable concentration in the solution. Stomatal uptake, when it occurs, is rapid; cuticular uptake is slow and continuing (16, 22, 44).

Open stomata are penetrated easily by vapors (58), or by oils (89, 106, 128, 165), not (or only slightly) by water, and to varying degrees by aqueous solutions containing surfactants. Ethanolic solutions apparently penetrate (88, 97). Completely closed stomata can exclude all fluids (164). Solutes dissolved in guttation droplets are thought to be carried in through stomata under certain conditions (7, 146).

Internal cuticle. Once in the substomatal cavity, there is still a lipid layer impeding movement, the so-called internal cuticle (4, 78, 141). This layer is thinner than the external cuticle. Its nature and permeability are less well understood. Histochemical tests indicate a suberin-like nature (137, 141). Plasmodesmata are believed to be absent in walls exposed to intercellular spaces (140).

Hydathodes, lenticels. There is scanty information as to the amount of penetration through hydathodes; probably they support a limited amount of entry (182) and are to be compared with small open stomata. Entry of P^{32} via lenticels and cracks of bark has been observed (76).

Effect of hairs. Hairs represent increased epidermal (or cuticular) absorbing surface (19). Pubescent leaves retained a greater amount of 2,4–D (with or without carbowax) than glabrous leaves (51). However, pubescence did not appear to be a factor in NAA penetration of apple leaves (75).

MOVEMENT IN THE LEAF

The following possibilities exist for the movement of spray substance from leaf surface to the phloem or xylem.

a) Across cuticle, then 1) anticlinal walls of epidermis and mesophyll, especially those of the bundle sheath extensions, or 2) via plasmodesmata in the outer periclinal epidermal wall, and symplast (interconnected protoplasts) to the phloem, or 3) a combination of 1 and 2.

b) Through stomata, then 1) across internal cuticle, then wall channels, 2) across internal cuticle, then into plasmodesmata and symplast, 3) in intercellular spaces, spreading along wall surfaces, or in bulk, to vascular bundles, 4) combinations of these.

[3]Foy, C. L. Studies on the absorption, distribution, and metabolism of 2,2–dichloropropionic acid in relation to phytotoxicity. Ph.D. Thesis, University of California, Davis. 1958.

Tissue path. The tissues involved in the movement of solutes from cuticle to phloem are not well known. By staining procedures a continuous avenue of pectinaceous substance extending from the outer cuticular surface along vein extensions to veins was found in apple leaves (126). In other studies the penetration of sodium dimethyldithiocarbamate was revealed by reaction with ferric sulfate introduced in the transpiration stream (121). Wylie (179) followed the transpiration stream by histochemical means, and found that it moved from the xylem preferentially along the bundle sheath extensions [cf. Esau (52)] to the epidermis, by-passing the palisade and spongy mesophyll cells. Earlier, LaRue (94) had come to similar conclusions. Movement of herbicides in the reverse direction along such a supplementary conducting system may occur (164). The bundle sheath is considered to be relatively hydrophilic (8). Leonard (96) suggests that "other things being equal, plants with the largest veins should be the most sensitive to high concentrations of 2,4–D."

Intercellular spaces. Movement of aqueous solutions in intercellular spaces is believed not to occur, or to occur only slightly. Surfactants increase the movement (98, 164). Benzene, chloroform, and olive oil move readily; paraffin oil moves more slowly (96). Oils applied to shoots can move systemically to the root through the intercellular system (105, 106), and can carry solutes such as 2,4–D in mass flow (124). Volatile liquids and subliming solids are distributed rapidly as vapor. The intercellular system, for stomatal-absorbed solutions, can accommodate a large reservoir of chemical and offers an extensive surface for cellular uptake. The ratio of internal surface to external surface can be as much as 31 to 1 (161). Passage across the internal cuticle must be similar to but more rapid than passage across external cuticle.

Cell walls. Cellularly, the movement of spray substance may be in the wall or in the protoplast. Using a fluorescent dye, sodium 3–hydroxy–5,8,10–pyrenetrisulfonate (PTS), which is non-toxic and not absorbed by normal uninjured protoplasm, wall movement is clearly shown in both upper and lower epidermis (19, 44). This movement is similar to the extrafasicular transpiration stream studied by Strugger (152) and by Wylie (179), but in the reverse direction. Fogg (58) concluded that DNOC moved in the leaf by diffusion in cell walls. The inward wall movement should be favored by coverage of the leaf surface by spray, or by conditions favoring a low transpiration rate. Movement in the walls may be controlled by the protoplasm [cf. Hülsbruch (86)], or it may be a physical diffusion in the pores of the wall substance. Cell walls are porous structures and generally are freely permeable to dissolved substances. Even large molecules, e.g., sucrose, pass through with ease. The suggestion has been made that callose formation, which can occur rapidly in pits of parenchyma cells under certain conditions, may interfere with parenchymatous transport by constricting plasmodesmata (38). Since whole walls can become callosed, wall transport might be inhibited, assuming that callose is a substance denser than

normal wall substance. Movement of uranin (sodium fluorescein) in cell walls of *Elodea* leaves is inhibited by ether narcosis and by plasmolysis, both reversible processes (151), suggesting that something besides simple diffusion or flow in pores is involved.

Symplast. Symplastic movement is under more direct metabolic control than is movement in the apoplast. This path has not been demonstrated for herbicides in leaves, although the usual requirement for an herbicide is that eventually it must react with protoplasm to produce a toxic response. Whether parenchyma tissue possesses a metabolic transport system for these foreign molecules remains to be demonstrated. Other substances, such as asparagine, are thought to move in the symplast (3).

Molecules entering the symplast must permeate the ectoplast (outer plasma membrane). The ectoplast exerts control on substances entering or leaving the cytoplasm. Once within the cytoplasm protoplasmic streaming could provide an accelerated cell to cell transport.

From morphological studies (20, 139, 153) plasmodesmata can be visualized as preferential intercellular transport channels, but again there is little proof that this in indeed true. It is possible that solutes could move symplastically from ectodesmata to sieve element, but there is evidence also for cell wall movement.

Syndermoplast. This term is employed by Hülsbruch (86) to describe a combined wall-protoplasm transport. She strongly favors the concept that all movement of substances in parenchyma tissue is controlled by the protoplasm.

On the basis of autoradiography it has been suggested that maleic hydrazide (MH) may follow an aqueous route to the phloem, and 2,4–D a more lipid one (28, 33). The paths are not known; possibly they are represented by walls and protoplasm respectively.

Entrance into conducting elements. Structure of the bundle sheath varies from species to species. There is no information as to the relative importance of wall and symplastic movement. Bundle sheath cells are rich in phosphatases so that active transport systems may exist for sugars at least (61, 169). Barrier and Loomis (9) suggest that a metabolically supported "loading" of herbicide in vein parenchyma cells is related to the mechanism of phloem transport.

In the typical dicot leaf the xylem lies on the upper (adaxial) side of the bundle, and spray substance should reach it first. This does not always occur; judging from macro-autoradiograms the amount of xylem absorption seemed to vary with species, conditions, and with type of herbicide employed (33). Distribution of labeled monuron [3–(*p*–chlorophenyl)–1,1–dimethylurea] within treated bean leaves was relatively rapid compared to export to other plant parts (54). The role of the xylem parenchyma in vessel uptake is not known. Once in the vessels the transpiration stream can carry the substance throughout the leaf to transpiring surfaces rapidly, and enroute possibly transfer it to the phloem or other tissue—bundle sheath, mesophyll, etc.

A similar lack of knowledge exists in relation to absorption of

solutes by sieve elements. Pressure flow movements and simple diffusion seem unlikely (24). Active processes are believed to support secretion of sugars or sugar phosphates across membranes of surrounding parenchyma cells [cf. review by Esau, et al. (53)]. That movement into and through the phloem may be a limiting or critical factor in systemic dalapon (2,2–dichloropropionic acid) toxicity is indicated by results with quackgrass (132). Although initial foliar uptake was detectable within 5 minutes, and a lethal quantity penetrated in 30 minutes, 3 hours were required for movement to rhizome or untreated shoot.

There is evidence that xylem-phloem and phloem-xylem interchange occurs readily (21, 103, 150).

Export by leaves. Export of chemicals by leaves is believed to require uninjured sieve tubes. The mechanism is unknown (53), although metabolic processes may be involved (10). Export of 2,4–D is favored by production of assimilates (170) and by metabolic and growth processes that provide "sinks" in other parts of the plant (29). Export from the leaf via the xylem is believed rare, occurring only when the diffusion pressure deficit of the water in the leaf environment is approximately zero, and when other leaves are transpiring and creating a tension in the xylem sap (31).

FACTORS INFLUENCING PENETRATION AND MOVEMENT

Penetration is influenced by plant factors, environmental factors, composition of the spray solution, and methods of application. All constitute variables that must be considered in any experimental approach.

Plant factors.

Character of the cuticle and internal cuticle. Probably a direct relation exists between thickness of cuticle and amount of penetration of 2,4–D (85), H_2SO_4 (131), oils (89), berberine sulfate (149), and urea (22). Light and low humidity increase thickness (45). Polar solutes permeate the cuticle more slowly than apolar solutes where molecular size is not too much a factor. Species differences in the nature of the cuticular surface are reflected in the wettability of leaf surface.

Distribution and size of the stomata. Deposit of spray on stomata-bearing surfaces is desirable, where stomatal penetration is indicated. Most leaves bear stomata on both upper and lower surface, but more on the lower (47). For some species, e.g., oaks, stomata are limited to the lower surface. The importance of sunken stomata and other morphological modifications in foliar penetration cannot be assessed at present. Stomatal size may be directly correlated with amount of stomatal penetration.[4]

A favorable water balance enhances absorption and movement of herbicides in several ways: hydration of cuticle (174), photosynthesis and phloem transport, enzymatic reactions, and growth (33, 95).

[4]Dybing, C. D. Foliar penetration by chemicals. Ph.D. Thesis, University of Calif., Davis. 1958.

Leaf morphology is important in relation to spray retention and placement. As expected, horizontal leaves retain more spray than vertical leaves (51), rough hairy surfaces more than smooth. For leaves with pubescence and ridges, as in grass species, air is trapped between leaf surface and spray drop. Rolling of leaves by plants exhibiting a water deficit results in decreased absorption of herbicide (58). Other things being equal, placement should be made over veins, and on areas having thin cuticle and numerous stomata.

Stage of growth. Young leaves are more penetrable (22, 91, 174). Sprays of 2,4,5–T were more effective on mesquite 90 days after the first leaves emerged in the spring (56). There are seasonal variations in the movement of 2,4–D in woody plants (97).

Injury. Increased penetration associated with leaf necrosis caused by toxic additives (see Surfactants, Miscellaneous additives), and by MCPA (2–methyl–4–chlorophenoxyacetic acid) has been reported (82). Leaves apparently attacked by aphids were more penetrable by MCPA (82). Plants sprayed with 2,4–D and run over by the wheels of the spray rig showed evidence of increased penetration (122). Damaging of hairs with a soft brush markedly increased the penetration of urea into tobacco leaves (167).

Metabolism. Herbicides may be "loaded" (9) or "accumulated" (33) in plant cells, particularly in parenchyma cells of the veins. Such a process would effectively increase the concentration gradient extending to the leaf surface. Production of photosynthate and stimulation of movement via the phloem of 2,4–D (39, 68, 79, 99, 108, 129, 170, 173) may steepen absorption gradients. Depletion of leaf carbohydrates decreased translocation of 2,4–D and P^{32} but not foliar penetration of these substances (9). A Q_{10} value of 2 for foliar uptake of 2,4–D (147) and a value of 2.6 for DNC (58) suggested limiting enzymatic reactions. The complexity of the overall penetration process makes it difficult to determine just where the temperature effect is exerted. It may lie in the accumulating process or in some permeability factor.

Environmental factors.

Light promotes penetration directly by stimulating opening of stomata, indirectly by supporting photosynthesis.[4] Instances of equal or greater absorption in darkness, compared to light (123, 160, 167, 174) could be attributed to other factors such as higher humidity and lower temperature. Evidence for the effects of light on permeability of cuticle and protoplasm cannot be assessed at present. Mitchell and Brown (108) early found that 2,4–D was not translocated from leaves of young bean plants that had been depleted of carbohydrates by storage in the dark. A photoperiod of 16 hours was more favorable for 2,4–D toxicity than shorter periods (41).

Warm temperatures, if not excessive, promote penetration through effects on: physico-chemical processes—increased rate of diffusion, lowered viscosity, etc.; physiological factors—acceleration of photosynthesis, phloem translocation, accumulation processes (see under Metabolism), protoplasmic streaming, and growth. Increased penetration of 2,4–D (9, 123) and of NAA (75) occurred with increasing

temperature. Mineral absorption and transport by leaves of bean and tomato increased from 50° to 70° F. (159); the effect was believed to be mainly on transport. High (90° F.) temperature, with relative humidity 35–40 percent, depressed absorption (based on percent kill) of potassium cyanate, due to increased evaporation of spray droplets (2).

A relatively high humidity prevents water stress in the plant, delays drying of spray deposit, favors stomatal opening, and may increase cuticular permeability. Penetration is shown to cease on crystallization of phenoxyacetic acids in spray droplets (82, 123). The apolar wax units in the cuticle conceivably are pushed farther apart by increased hydration of the cutin matrix (164). Absorption and translocation of 2,4–D were enhanced by high humidity, and these effects were directly correlated with degree of stomatal opening.[5] Penetration of MH is promoted by high humidity (181). On the other hand, uptake of sucrose by tomato leaves was found to be independent of humidity levels (175). Extended dry periods may increase cuticular thickness with an accompanying lower penetrability.

Rain, if it occurs soon after spray application, can wash off spray deposits and reduce penetration (171, 181).

Effect of spray drop itself. A spray droplet prevents transpiration in the area covered, and it is proposed that a reversal of the transpiration stream occurs in this region to move spray substance into the leaf. This emphasizes a disadvantage of too rapid evaporation of the drops and subsequent crystallization of the herbicide. Stomatal closure due to growth regulator application has been reported (55).

Formulation.

Structure of toxicant. Various 2,4–D formulations gave increased effectiveness in the order: Na salt, NH_4 salt, amine, ester (76). Structure-activity relations have been studied in certain groups, for example, the phenoxy derivatives (40, 82, 113, 166) and the N-substituted alpha-chloroacetamides (72). The influence of slight structural changes on activity is sometimes striking. Penetration may be a controlling factor in these changes but there is little direct evidence that this is true (172).

In general, apolar molecules are thought to enter leaves more readily than polar molecules (23). Penetration of growth regulators may be improved when the molecule exhibits the proper polar-apolar balance (163, 166). The same holds true for absorption of toxicants by fungi (125).

pH and buffers. The pH and buffer composition of herbicidal solutions are important factors in effectiveness (73, 101). Many weak acids penetrate cells more readily in the undissociated form, i.e., at relatively low pH values (1, 25, 32, 154). Sorption by cuticle is markedly

[5]Pallas, J. Effects of temperature and humidity on the absorption and translocation of 2,4–dichlorophenoxyacetic acid and benzoic acid. Ph.D. Thesis, University of Calif., Davis. 1958.

influenced by pH and by type of buffer (119, 120). Penetration of 2,4–D was better at pH 4 than at pH 7 (26, 74); MH penetrated best at pH 7 (30). The buffering effect of the leaf on single droplets may be considerable (143). Different cations may affect the rate of anion penetration independent of pH (90, 120). Both NH_4^+ and $PO_4^{\equiv}$ increased 2,4–D penetration at pH 5 (158).

Surfactants. It has long been known that surfactants increase herbicidal effectiveness (13, 15, 29, 34, 35, 49, 59, 66, 71, 77, 93, 96, 97, 110, 111, 112, 145, 147, 148, 160). They also enhance the action of defoliants (17). The most efficient surfactants were reported to increase plant response to 2,4–D about 14-fold (174). Use of radioactive herbicides has revealed striking surfactant effects on penetration (48, 59).

The response to surfactants may be due to one or more of the following: a) improving coverage; b) removing air films between spray and leaf surface; c) reducing interfacial tension between relatively polar and apolar submicroscopic regions of the cuticle; d) inducing stomatal entry; e) increasing the permeability of the plasma membrane, through stimulation or incipient toxicity; f) facilitating cell wall movement in the region of the wall-cytoplasm interface; g) acting as co-solvents; h) interacting directly with the herbicide in some manner; i) acting as humectants secondarily. Van Overbeek and Blondeau (165) considered hydrocarbons and surfactants to "solubilize" into the plasma membrane, displacing the fatty molecules and "opening up" the membrane. This hypothesis could explain permeability increases.

The action of a surfactant may vary depending on the nature of the penetrating solute. For example, Tween 20 was ineffective in phosphorus (P^{32}) penetration into bean leaves (159), but increased the response of maize plants to foliar-applied gibberellins 10-fold (114). Triton X–100 improved phosphorus absorption but depressed magnesium absorption (57). Other influencing factors, of course, are not excluded.

Concentration of surfactant increasing up to a point gives progressively increased penetration. For some compounds this point may be in the range of 0.1% to 0.5%. Beyond this there may not be an additional effect. One reason may be micelle formation by the surfactant molecules themselves (104). The optimum concentration of Tween 20 in 2,4–D sprays varied with the concentration of 2,4–D (96).

Surfactants vary in their capacity to improve penetration. The relation between molecular structure and effectiveness of these substances is a problem of immediate interest. At present only generalizations can be made: the molecule should markedly reduce the interfacial tension between plant lipid and water, and, for oil soluble herbicides, between water and oil carrier as well; it should be soluble in both water and apolar solvents; it should not be excessively toxic, although in some instances some toxicity may be desirable (36, 81). Some of the most widely used surfactants are those having a degree of toxicity (63). It is, of course, recognized that toxic sub-

stances, whether herbicide or additive, can block phloem translocation if the concentration is sufficiently high (96, 97, 180). Mankowich (102) studied the physico-chemical properties of surfactants, finding direct relationships between wetting (on basis of contact angle) and both surface tension and interfacial tension. Surface tension lowering of water is not in itself sufficient to explain the beneficial action of surfactants in herbicidal solutions (59, 107, 174). Addition of Tween 20 markedly enhanced the phytocidal activity of alcoholic solutions of 2,4–D while there was no effect on surface tension (174). The spreading coefficient as a measure of wetting ability, using paraffin as a model of lipid surface, was not well correlated with the toxicity of a number of phenoxy compounds (40). Wetting is important, but it must be recognized that spray load on some leaves can actually be reduced by the use of wetting agents (14, 90, 159). The thinner adherent films also dry more rapidly (90).

Whereas surfactants usually enhance foliar penetration of herbicides, they have performed variously with phosphate, either increasing (57) or decreasing (9, 90, 154, 159) absorption. Complexing (and thus inactivation) of surfactant by phosphate may occur (9, 90, 154). The problem needs further study, with attention to structure and concentration of surfactant, method of application, and component interaction.

Humectants improve penetration by preventing drying of spray deposit, thus assuring an extended penetration time. Examples are: carbowax, for 2,4–D (50, 123); glycerine, for 2,4–D (83), for phosphorus (57), for MCPA (82), for streptomycin (67), for MH (181); propylene glycol for MH (30); and molasses for DNOC (58). They also may modify the permeability of the cuticle in some way (144, 181).

Deposit builders. Increasing the spray load—the external reservoir—has been given insufficient attention. It is realized that incorporation of bulky colloidal materials to the spray presents problems in application. Care must be taken also to prevent clogging of stomatal openings where this may be important.

Cosolvents. The advantage of polyethylene glycols as cosolvents, spreaders, and humectants, was recognized early (110). Other materials find use in commercial formulations.

Oils and emulsions. Oil solvents can increase the penetration of 2,4–D (26, 49). Toxic oils, such as diesel oil, may provide poor kill due to phloem inactivation (96). Oil-water-surfactant emulsions facilitate a uniform application of water-insoluble herbicides; evaporation of the water leaves an oil non-volatile residue. The oil component aids in wetting and doubtless acts as both a cuticular and a stomatal penetrant. A slight toxic action also may increase permeability of the protoplasm (37, 165). Oils were superior to oil-water emulsions in 2,4–D and 2,4,5–T applications to bean (96). Oil emulsions of 2,4–D salts are more effective than aqueous solutions (66, 84, 180). However, no improvement of emulsions over aqueous solutions was found in mesquite control (56). Silicone oils penetrate

stomata more rapidly than petroleum oils (96), but the non-toxic forms are notably poor solvents for herbicides.

Miscellaneous additives. Sucrose aids foliar absorption of growth regulators (87, 108, 173) and of cobalt^{60} (68). It behaved oppositely in P^{32} penetration (159). Sucrose absorbed by leaves replaces photosynthate and enhances translocation of 2,4–D downward (79, 129). Boron was found to improve penetration and movement of sucrose (64), of 2,4–D (65, 109), and of glucose (115). However, it is questioned whether boron additives will prove helpful in the practical application of 2,4–D (96). Urea increased the rate and total absorption of 2,4–D (156). Various toxic adjuvants enhance the penetration of 2,4–D presumably by increasing permeability (81). A mixture of substances [$CuSO_4$, $Zn(NO_3)_2$, Fe-citrate, $Mn(NO_3)_2$, and $H_2B_4O_7$] added to a 2,4–D spray reduced the toxic response (157), but it was not possible to separate absorption factors from other effects. Ethanol improved the absorption of 2,4–D acid but not of the amine salt (96).

Methods of application.

Close high-pressure spraying may result in increased stomatal penetration (if stomata are open). Full coverage of the leaf should be superior to droplet application, if drying time is not a factor. Spraying the lower leaf surface should be superior to upper surface, where this is feasible. Spraying in the morning when stomata are open should be superior to late afternoon or evening. Droplet spacing studies indicate a maximum distance of 3.1 mm necessary to maintain a high level of herbicidal (2,4,5–T) effectiveness (11).

PROBLEMS

In conclusion, the authors present in summary form several problems that need study in connection with foliar penetration of herbicides, and, for that matter, of all leaf-applied chemicals. It is apparent from the foregoing that little is known with certainty.

1. As a firm basis to support research in spray formulation and application, it is necessary to study and understand the pathways and mechanics of movement into and within the leaf, and systemically out of the leaf. As specific examples, the meaning of plasmodesmata, the mechanism of cell wall transport, guard cell action, and "accumulating" processes, may be mentioned.
2. Since systemic distribution usually is critical for herbicidal effectiveness, elucidation of mechanisms of absorption by xylem and phloem elements of the leaf blade are of special importance.
3. It is essential to know the effect of each of the important environmental factors on the path, mechanics, rate, and volume of transport.
4. Improvement in formulation doubtless will supply the most immediate means of increasing penetration. In the authors' view the ideal formulation is one that will penetrate stomata immediately, but one that will also provide a relatively large external reservoir, the thick deposit remaining liquid or at least plastic, not interfering greatly with gaseous exchange, and containing substances that im-

prove stomatal and cuticular penetration—surfactants, penetrants, and possibly stimulants if suitable compounds can be found.

5. Comparative studies of structure and function of the leaves of a wide variety of weed and crop plants are necessary, because the species variable confounds all other variables in the penetration complex.

6. In addition to estimating penetration indirectly on the basis of subsequent injury or growth response, more quantitative and direct determinations are needed. Distinction between absorption and translocation processes then will be clearer.

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A Fluorescent Dye Method for Foliar Penetration Studies[1]

C. D. Dybing and H. B. Currier[2]

Foliar application of herbicides, plant regulators, and nutrients is a well established practice. Yet, the many contradictory reports in the literature (7) indicate that the routes of entry into and movement within leaves are not clearly known. This situation has probably arisen because presently available techniques do not precisely establish the location of the penetrant on the cellular level.

Histochemical techniques have been employed with great success by Knight et al. (10), Rohrbaugh (14), Rice and Rohrbaugh (13), and others to demonstrate the sites of entry and routes of movement of oils. In the case of aqueous solutions, on the other hand, most evidence is indirect in nature, and histochemical evidence is limited to very few reports such as those of Palmiter et al. (12) and Wylie (24) for penetration and movement of iron. Many workers (3, 5, 15, 21, 22, 23) have compared stomatal with cuticular penetration by such indirect methods as measurement of plant response to growth regulators or nutrients, autoradiography or counting of tissues containing radioactive tracers, or measurement of nonabsorbed chemical. The importance of stomatal entry has not been clearly established by these methods. More direct evidence is obtained by using other techniques. For example, Aslander (2) and Crafts (6) studied the penetration of dilute sulfuric acid solutions by microscopic detection of injury resulting from the toxic action of the penetrant; however, conflicting observations were reported. Autoradiography of leaf sections has been used by Gustafson and Schlessinger (9) to demonstrate penetration of cobalt^{60} into bean leaves in the dark, but to date this promising method has not been widely applied in attempts to determine the route of penetration.

Because most of the conventional methods yield only indirect evidence, a more direct method involving a fluorescent dye has been tested. This type of tracer was used to study foliar absorption of dew water by Steubing (18), who followed entry and movement of berberine sulfate with the fluorescence microscope. By a similar method, Butterfass (4) observed foliar penetration via the club-shaped hairs of *Vicia faba* L. and *Phaseolus coccineus* L. Orgell[3] used both fluorescent and nonfluorescent dyes to study penetration of isolated plant cuticles. In the work reported here, the fluorescent tracer method has been considerably expanded and improved for application to the problem of foliar absorption of aqueous solutions.

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[3]Orgell, W. H. The isolation and permeability of plant cuticle. Ph.D. Thesis, University of California, Davis. 1954.

METHODS AND MATERIALS

The fluorochrome used was sodium 3–hydroxy–5,8,10–pyrenetrisulfonate, a dye used by Strugger (19) in his studies of the extrafascicular transpiration stream in plants. Of the 14 dyes tested, this compound exhibited the most suitable properties for use as a fluorescent tracer. Test solutions usually contained 0.1 percent dye; in many tests the wetting agent Vatsol OT (sodium dioctylsulfosuccinate) was included at concentrations ranging from 0.01 to 0.5 percent. The test plant, except where otherwise noted, was *Zebrina pendula* Schnizl. which has large stomata mostly restricted to the lower surface (8).

The dye solutions were usually applied by immersion of all but the cut surface of detached leaves. Droplet application gave similar results. Nonabsorbed dye was removed at the end of the test period by washing in running water for 1 to 3 minutes. Tests showed that all dye was removed from the surface in the first minute of washing; no leaching of penetrated dye was apparent. After being washed, the leaves were blotted and observed in ultraviolet light provided by two Projector lamps (Stroblite Co.) with H100 SP4 spot type mercury arc bulbs (GE). Most of the visible light produced by these lamps was excluded by No. 41 UV glass filters (Kopp Glass Inc.) with a peak transmission at about 366 mμ. Results were recorded by photography, a Wratten 85 filter being placed in front of the camera lens to eliminate ultraviolet radiations from the light striking the film. For photomicrography the leaf was held on the stage of a microscope and illuminated from above by the two lamps. Spurr (16) gives further information on fluorescence photography and equipment.

To obtain leaves with open stomata, collections were made in the field during the day or from greenhouse plants held in a light chamber. Collections made at night or from plants held in a dark chamber provided leaves with closed stomata. The stomatal condition at the time of treatment was determined by microscopic examination.

In the final experiment a radioactive tracer was used to check the accuracy of the fluorescent tracer method. *Zebrina* leaves were treated with 5-μl droplets of a solution containing 0.5 percent dye, 0.1 percent Vatsol OT, and 0.05 μc C^{14}-labeled maleic hydrazide. After five minutes the nonabsorbed material was removed by washing as described earlier. The distribution of the fluorescent tracer was recorded photographically. Then the leaves were oven-dried at 60 to 65° C, and the location of the radioactive tracer was determined by autoradiography.

RESULTS

In many tests, absorption of aqueous solutions was greatly enhanced by addition of various surfactants. An example of such a test is shown in Figure 1A. The treatment was immersion of detached *Zebrina* leaves for 5 minutes in dye solutions, some of which contained Vatsol OT. The surfactant greatly increased penetration except at the lowest concentration, 0.01 percent. The untreated control leaf is visible in the photograph because of its primary fluorescence. The dye solution containing no wetting agent did not pene-

Figure 1. Penetration of fluorescent dye solutions into *Zebrina* leaves. A. Left to right: untreated control, dye alone, dye plus Vatsol OT at 0.5, 0.1, 0.05, and 0.01 percent respectively. All treatments were for 5 minutes. Lower surface of leaves is shown. B. Left to right: untreated control, two leaves treated for 30 seconds, and two leaves treated for 10 seconds. Wetting agent included in test solution. Stomata open.

trate in this 5-minute treatment, and in other tests such solutions have not been absorbed to any appreciable extent by *Zebrina* leaves in 30 minutes. In all cases where the surfactant was included in the test solution, penetration of the lower surface was much more rapid than that of the upper. In addition, the dye solution entering the lower surface moved through the leaf via the walls and reached the upper surface in 10 to 20 minutes.

In early tests with this method, penetration was slow in some cases and rapid in others. In attempts to determine the cause of this vari-

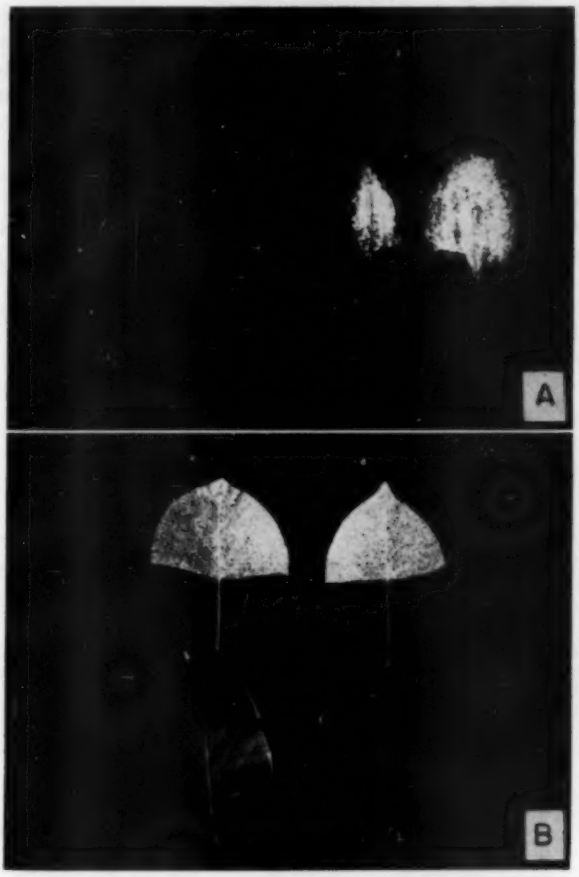

Figure 2. A. Two-minute treatment of *Zebrina* leaves with dye plus Vatsol OT. Left to right: two leaves with stomata closed, untreated control leaf, and two leaves with stomata open. B. Same as A except pear leaves used and only apical half of leaves immersed. Upper: leaves with open stomata; lower: stomata closed.

ation, a correlation was sought between degree of stomatal opening and amount of penetration. Figure 1B shows that with *Zebrina* some penetration occurred even in 10 seconds when leaves with open stomata were immersed in dye solution containing 0.5 percent Vatsol OT. In other experiments, penetration into leaves with stomata apparently uniformly closed was compared with penetration when the stomata were mostly open. The treatment time was 2 minutes, and wetting agent was added in all cases. Figure 2 shows the results obtained with *Zebrina* and pear (*Pyrus communis* L.). Rapid penetration occurred only when the stomata were open; closed stomata

apparently prevented entry. The same results were obtained with leaves of cotton (*Gossypium hirsutum* L.), Red Kidney bean (*Phaseolus vulgaris* L.), dandelion (*Taraxacum vulgare* (Lam.) Schrank.), apricot (*Prunus armeniaca* L.), grape (*Vitis vinifera* L.), and plum (*Prunus* sp.). Similar comparisons have not been made for aqueous solutions lacking surfactant.

Figure 3A shows a photomicrograph of a leaf immersed for 5 minutes in dye solution containing wetting agent, and Figure 3B shows a 30-minute application. In the shorter treatment the dye accumulated only in the substomatal chambers, while the longer treatment

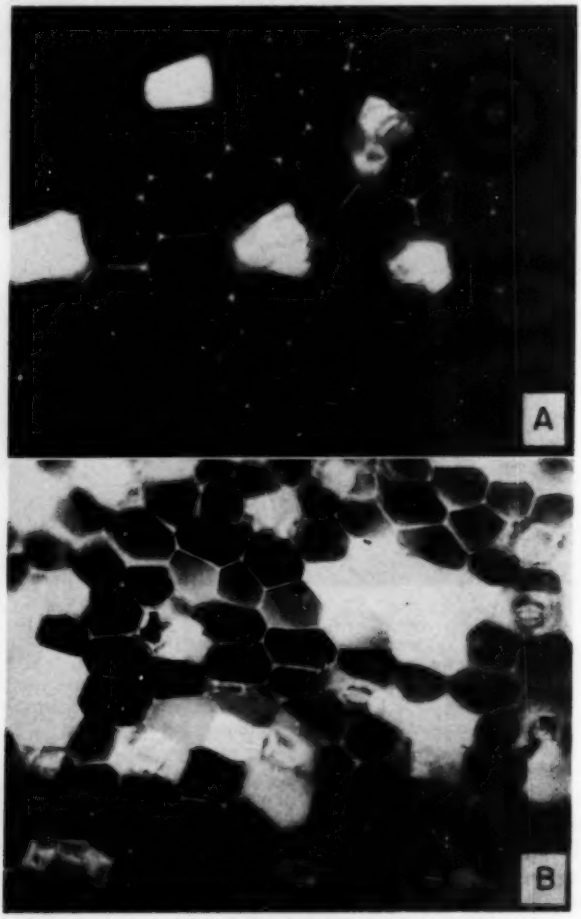

Figure 3. Photomicrographs showing dye in *Zebrina* leaves. In A, leaves were immersed for 5 minutes; only substomatal chambers contain dye. In B, the treatment was 30 minutes, and dye is present in substomatal chambers, anticlinal walls, and some epidermal cells. Surfactant was included in both tests.

resulted in staining of the epidermal cells as well. The presence of varying amounts of dye in substomatal chambers was taken as further evidence that open stomata participated in the initial rapid penetration observed in the tests described earlier. The anticlinal epidermal walls are visible in Figure 3A mostly because of their blue-white primary fluorescence; little or no dye from the applied solution is located in them. On the other hand, the anticlinal walls in Figure 3B do contain dye. Although these two wall fluorescences are sometimes difficult to distinguish in photographs, they are readily separable by microscopic examination because the secondary fluorescence induced by the dye has a distinctive yellow-green color as well as greater intensity. Staining of anticlinal walls was the main evidence of cuticular penetration of the nonstomatal upper surface. The fluorescence of entire epidermal cells in the longer treatments is of interest since Strugger (19) has indicated that the dye used in these studies does not enter living protoplasm. The dye is relatively nontoxic to onion bulb scale epidermal cells, for 80 percent or more of the cells retained the capacity for plasmolysis after 7 days immersion in 0.5 percent dye solution. Vatsol OT was highly toxic at concentrations above 0.05 percent. Hence the fluorescence of the epidermal cells in Figure 3B could be accounted for by surfactant toxicity and the resultant entry of dye into the protoplasts.

Since most foliar-applied herbicides are molecules which are quite different from the large, polar dye molecule, it is necessary to correlate dye results with other penetrants. Results of one such comparison between tracers are shown in Figure 4. Radioactive MH and the fluorescent dye were applied in the same drop to the lower surface of *Zebrina* leaves, and the distributions of the two tracers were determined as described in the section on methods. The fish-

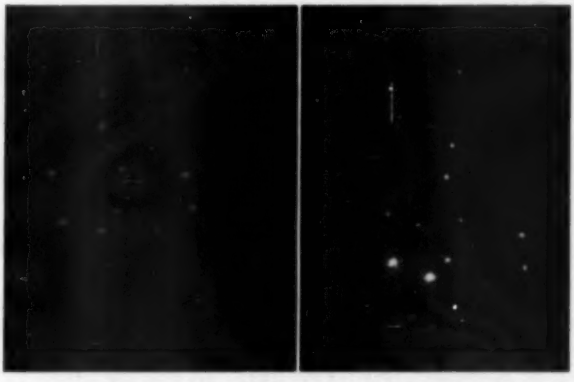

Figure 4. Penetration of fluorescent dye compared with that of C^{14}-labeled maleic hydrazide. Test solution contained 0.5 percent dye, 0.1 percent Vatsol OT, and 0.05 μc MH* per 5-lambda drop. Five-minute treatment. On left is view of leaf showing distribution of dye, and on right is autoradiogram of C^{14} distribution (ca 5 ×).

hook-shaped pattern obtained in both cases shows that the two tracers were at the same location in the tissue 5 minutes after application. This perfect correlation was observed in 12 replications. Other tests with radioactive tracers also support the accuracy of the dye method. Specifically, maleic hydrazide with surfactant penetrated *Zebrina* leaves in 5 minutes, but no penetration occurred when the surfactant was omitted. In addition, 3–amino–1,2,4–triazole with 0.1 percent Vatsol OT penetrated sufficiently in 30 seconds to give strong autoradiograms on Kodak no-screen X-ray film after 7 days exposure.

DISCUSSION

From these tests the fluorescent tracer method appears to be a useful tool for foliar penetration studies. The method as outlined is simple and does not require expensive equipment. Another very important advantage is that the portals of entry can be established because the location of the absorbed dye can be readily determined microscopically. Our tests with radioactive tracers have given support to the observations concerning initial penetration of aqueous solutions obtained with the fluorescence method. However, since the dye employed is limited to the walls of normal uninjured cells, internal movement of this tracer may not be the same as that of other foliar absorbed chemicals. The fluorescence method should prove useful in following stomatal penetration, in screening anionic and nonionic surfactants, and in studying modes of action of surfactants. In addition the dye may serve as a criterion of vitality in studies dealing with the effect of surfactant toxicity on penetration.

The observed increase in penetration resulting from addition of surfactant is in agreement with many reports (3, 5, 11, 17, 23). However, in a few instances (3, 20, 21) no such increase was observed in absorption of various inorganic radioactive tracers.

The present tests provide evidence that the stomata are involved in penetration of aqueous solutions containing surfactants: entry is very rapid only when stomata are open and the substomatal chambers become filled with dye. Without a surfactant, foliar penetration is much slower, but evidence is insufficient to rule out stomatal entry of these solutions. As indicated earlier, the question of stomatal penetration has been widely discussed in the literature. The fluorescent tracer method may provide the direct evidence necessary for an adequate solution of this problem.

Movement of fluorescent dye through the walls emphasizes the possibility that this pathway may be of major importance in passage of a chemical within the leaf from the site of initial entry to the conductive tissue. Arisz (1), however, does not believe that this is the major pathway for movement of KCl in *Vallisneria* leaves; symplastic movement is considered more likely in his view.

SUMMARY

A technique has been described for the use of a fluorescent dye in foliar penetration studies. The method is simple and rapid, requires

no expensive equipment, and establishes the location of the penetrant on the cellular level. Tests with radioactive tracers confirm the accuracy of the method.

In the application of the method, surfactants greatly enhanced foliar penetration. Penetration of the lower leaf surface was much more rapid than penetration of the upper. Solutions containing a surfactant apparently entered mainly by way of open stomata; closed stomata excluded these solutions. Stomatal entry was very rapid; cuticular penetration also occurred but was relatively slow. Dye solutions containing no wetting agent penetrated much more slowly, and the site of entry was not determined in these studies.

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The Control of Weeds in Newly Established Sour Cherries

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ONE of the factors to be considered in the establishment and maintenance of an orchard is the competition from weeds for the available supply of moisture and nutrients. Mechanical elimination of competitive plants is time consuming, expensive, and may not be done as early or as frequently as it is needed each year. The use of herbicides as a supplement to such a program now seems worthy of serious consideration. In some instances, as will be seen below, the use of mechanical methods may be eliminated altogether.

Early investigations on the use of chemicals under tree fruits were carried out with herbicidal oils. Frequently these were fortified with dinitro compounds such as 4,6–dinitro–*o*–*sec*–butylphenol (DNBP) or with pentachlorophenol (PCP). The use of these materials has been studied by Curtis (1). These chemicals will give temporary control and will often provide excellent top-kill of many weed species. They have three serious disadvantages: (1) If oils are used carelessly, the soft tissues of the bark will be injured, (2) they are quite ineffective for lasting control of perennial weeds which frequently become the real troublemakers under trees, and (3) they must be used repeatedly. Furthermore, the dinitros are extremely toxic, pentachlorophenol is very inconvenient to handle because of its irritation to the nasal passages, and oils create a serious fire hazard at all stages of the operation.

In 1952 Gilbert and Holm (3), and in 1954 Holm and Gilbert (6) reported on the use of the newer organic herbicides when used in sour cherries and apples. The urea herbicide 3–(*p*–chlorophenyl)–1,1–dimethylurea (monuron) was the outstanding chemical in these tests. Hall (4) working with pears and apples, and Day (2) working with orange, lemon, and grapefruit, have also reported the successful use of monuron for weed control without injury to the trees. Hemphill (5) in Missouri reported sporadic injury to young apple trees with low rates of monuron. 2,2–Dichloropropionic acid (dalapon) has also been used to control grasses under apples and pears, according to Hall (4) and Harrison (7).

The purpose of the experiments reported here was to select from the newer organic herbicides those which could be used safely for the control of annual and perennial weeds in young orchards. In 1954 the most effective chemicals were incorporated into a long-term experiment designed to study the response of the trees and weeds when treatments were repeated annually in the same blocks.

MATERIALS AND METHODS

This study was conducted in a Montmorency sour cherry tree block planted in 1951 at the Peninsular Branch Experiment Station,

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Sturgeon Bay, Wisconsin. All of the trees received an annual application of an N–P–K fertilizer to encourage optimum growth. A management system of clean cultivation with a winter wheat cover crop was followed. Beginning in 1953 an 8-foot square around each tree was left uncultivated. A standard insect and disease control program was followed throughout each growing season.

The weed population in the orchard consisted primarily of the following: *Agropyron repens*, quackgrass; *Echinochloa crusgalli*, barnyard grass; *Setaria* sp., foxtails; *Chenopodium album*, lambsquarters; *Amaranthus retroflexus*, pigweed; *Brassica* sp., mustards; volunteer clovers and alfalfa.

From 1951 through 1953, in several experimental areas, the following herbicides were tested in single applications, double applications, and in combinations:

Monuron (3–(*p*–chlorophenyl)–1,1–dimethylurea)
CIPC (isopropyl N–(3–chlorophenyl)carbamate)
TCA (trichloroacetic acid)
Endothal (3,6–endoxohexahydrophthalic acid)
Stoddard Solvent
Maleic hydrazide (1,2–dihydro–pyridazine–3,6–dione)
Sesone (2,4–dichlorophenoxyethyl sulfate)
DCU (dichloral urea)

From these preliminary studies monuron, CIPC, and a combination of monuron and CIPC were selected as having the best possibilities. These three treatments, together with weeded and unweeded blocks, were included in the long-term experiment which was initiated in 1954.

Treatments consisted of single row plots of nine trees per row with four replications. The herbicides were applied in 40 gallons of water per acre at 30 psi using fan-type nozzles. All chemical rates are given in pounds per acre of active ingredient. An area 5 feet wide was sprayed on either side of each tree using a bicycle-type sprayer. The spray was so directed as to strike the base of the tree trunk. All of the herbicides were applied in April of each year.

Five trees from each treatment row were selected on the basis of uniformity of growth as criterion trees for the collection of data.

In 1956 and 1957 all of the weeds in an area 8 feet square around the trees were cut off at ground level and weighed.

RESULTS

The average terminal growth of sour cherry trees was not affected by herbicides in any year. In fact, the terminal growth of the trees was remarkably uniform in an experiment which was quite sensitive as evidenced by the low coefficients of variability (Table 1). The reduced growth in 1956 and 1957 was associated with the production of substantial crops of fruit on the trees. These data, which represent the average of 10 terminals per tree for 1954–5 and 15 terminals in 1956–7 were examined statistically and no significant differences were found.

Trunk diameter measurements (4 inches above soil level) were made from 1955 through 1957 (Table 2). The herbicides had no significant effect on the increase in trunk diameter.

Table 1. Terminal growth of sour cherry trees.

Treatment	Appl. lb/A	Average growth per terminal inches			
		1954	1955	1956	1957
Control—unweeded		18.8	18.4	12.0	13.89
Control—weeded		18.2	19.5	12.9	12.75
Monuron	2.5	19.1	19.3	13.0	13.97
CIPC	20.0	19.0	19.9	13.5	13.77
Monuron plus CIPC	2.5 20.0	19.3	20.2	13.2	13.57
CV%		3.6	7.1	8.2	5.2
LSD 1% level		N.S.	N.S.	N.S.	N.S.

Table 2. Trunk diameters of sour cherry trees.

Treatment	Appl. lb/A	Average trunk diameter inches		
		1955	1956	1957
Control—unweeded		2.35	2.68	3.04
Control—weeded		2.64	3.05	3.46
Monuron	2.5	2.56	2.98	3.43
CIPC	20.0	2.52	2.94	3.37
Monuron plus CIPC	2.5 20.0	2.62	3.07	3.46
CV%		8.9	5.5	5.8
LSD 1% level		N.S.	N.S.	N.S.

In 1955, when the trees were still predominantly vegetative (year preceding the first heavy crop year), the area of 15,000 leaves from spurs and terminals was measured by use of an optical planimeter designed by Miller, et al. (8). The spur leaf samples consisted of all leaves from 10 spurs selected at random from two-year wood. Terminal leaf area was obtained by collecting the sixth leaf (from the tip) through the tenth leaf on five terminals selected at random throughout each tree. These data (Table 3) show that none of the

Table 3. Spur and terminal leaf area of sour cherries.

Treatment	Appl. lb/A	Spur leaf area sq in		Terminal leaf area sq in	
		1955	1957	1955	1957
Control—unweeded		94.6	75.0	113.5	106.2
Control—weeded		96.6	77.6	122.7	113.2
Monuron	2.5	96.5	75.8	120.7	110.0
CIPC	20.0	94.9	78.6	122.1	115.0
Monuron plus CIPC	2.5 20.0	91.4	74.4	122.0	112.6
CV%		5.0	6.8	3.9	6.4
LSD 1% level		N.S.	N.S.	N.S.	N.S.

chemical treatments significantly affected the growth and development of the leaves.

The number of spur leaves produced in these years was determined on the spurs from which leaf area samples were taken. The number of leaves per spur ranged from 5.0 to 5.3 among treatments for the two years. These data were analyzed statistically and no significant differences were found. The coefficients of variability are low for biological material of this type.

In 1956 the first yield data were obtained (Table 4). A lack of uniformity among trees was not unexpected in the first bearing year, but in this case no significant differences were found due to treatment. The weight of 200 cherries selected at random from each replicate was uniform, indicating that the herbicide had no noticeable effect on fruit size.

Table 4. Yield and size of sour cherries—1956.

Treatment	Appl. lb/A	Av. yield lb/tree	Fruit size wt 200 fruits grams
Control—unweeded		6.9	753
Control—weeded		8.4	769
Monuron	2.5	9.0	756
CIPC	20.0	7.2	776
Monuron plus CIPC	2.5 20.0	9.7	751
CV%		28.4	3.9
LSD 1% level		N.S.	N.S.

The control of weeds in the orchard during the 1956 and 1957 seasons was highly satisfactory following the treatments with monuron and the mixture of monuron and CIPC (Figure 1). The treatment with CIPC at 20 lb/A did not provide adequate weed control. The degree of weed control afforded by these chemicals has been studied each year in one or more experiments, in both apple and cherry orchards, since 1951. Following planting, where only normal cultivation was practiced, the weed population gradually increased. Where monuron was used alone, or in combination with CIPC, satisfactory weed control was obtained each year with no apparent injury to the trees. The general level of weed control for the aforementioned treatments has been consistent in the experiments conducted since 1951 and was at the level reported in Table 5. CIPC was somewhat variable in its control of weeds, but monuron has consistently given excellent results.

The rate of monuron (2,5 lb/A) was selected from preliminary trials conducted in 1951–52 which indicated that this amount was adequate to keep plots free of competing grasses and broad-leaved weeds. However, this rate was not high enough to eliminate serious infestations of established weeds.

A survey plot has been maintained since 1952 to ascertain the effects of repeated applications of high rates of monuron. This plot consists of four trees which have received 10 lb/A of monuron annually and four trees which have received 5 lb/A annually. After six

Figure 1. Upper. The normal stand of grasses and broad-leaved weeds following several seasons without cultivation or chemical treatment. *Lower.* The control of weeds where monuron was used at 2.5 lb/A.

Table 5. Control of weeds in sour cherries.

Treatment	Appl. lb/A	Percent weeds controlled (by weight)		Weed weights lb/tree	
		1956	1957	1956	1957
Monuron	2.5	100	100	0.02	0.05
CIPC	20.0	50	33	5.32	5.28
Monuron	2.5	94	96	0.71	0.33
plus CIPC	20.0				
Control, unweeded				12.64	7.79
CV%				18.00	32.10
LSD 1% level				1.93	2.47

years of treatment, the trees still appear normal during the early part of the growing season. By midsummer some trees in each treatment show the usual symptoms of leaf injury characteristic of the urea herbicides. The leaf injury on two of the trees treated with 10 lb/A has reached the point where it has limited terminal growth. The information obtained from this survey has been of considerable value for it has indicated that the 2.5 pound treatment, which has never given injury under annual treatment, should not present a severe toxicity problem. Should visual symptoms of leaf injury become apparent in future years, it is quite likely that the growth of the trees will not be impaired at this rate and treatments may be suspended for a year.

DISCUSSION

The soil conditions under which this experiment was conducted are quite different from those found in other sour cherry producing regions of the United States. The soil in the experimental area is a Longrie silt loam with an average pH of 7.5. The soil is also classified as a shallow to very shallow orchard soil since the depth ranges from 1 to 2 feet over a Niagara limestone bedrock. The average rainfall in the area is 28 inches with 50–55% falling during the 5 months, May to September.

The experiment was designed to study the effect of the herbicides on several phases of tree growth. The entire area was thus maintained free from all weeds until the trees had become well established. The data obtained since 1951 have thus far failed to show any harmful effects from monuron (2.5 lb/A) or CIPC, and as yet there has been no evidence of reductions in tree growth in the blocks where weed populations are building up. In order to obtain a more complete picture of weed competition effects in the future, the untilled area under the unweeded controls has been enlarged until it extends beyond the drip line of the trees.

In an area immediately adjacent, a cultural-moisture availability experiment has been under way since 1952 to study the effects of sod competition with cherries. It has already shown great differences in the ability of sour cherry trees to compete with a grass sod for the available moisture. Trees under clean cultivation have averaged 14.91 inches of terminal growth while the trees which are growing in an unmowed sod averaged 7.76 inches during the five-year period from 1953–57. The trees are now 8 years old. Total fruit yields per tree in the same plots were 28.32 pounds for the clean cultivation and 10.98 for the unmowed sod for the years 1954–56.

SUMMARY

1. Monuron, and mixtures of monuron with CIPC, have been used for the control of broad-leaved weeds and grasses in sour cherry orchards for 4 years. These treatments have given excellent weed control.

2. Detailed measurements of terminal growth, number of spur leaves produced, total leaf area on spurs and terminals, trunk diame-

ters, fruit size, and total yield of cherries revealed that the herbicides had no detrimental effect on the growth and development of trees following 4 years of continuous treatment.

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BRIEF PAPER

Metal Plot Stakes for Rocky Soils[1]

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FREQUENTLY, wooden plot stakes are difficult to establish and maintain in conducting weed control and other experiments on rocky soils. Metal plot stakes were developed especially for use on rocky ditchbanks of irrigation systems in the Pacific Northwest. However,

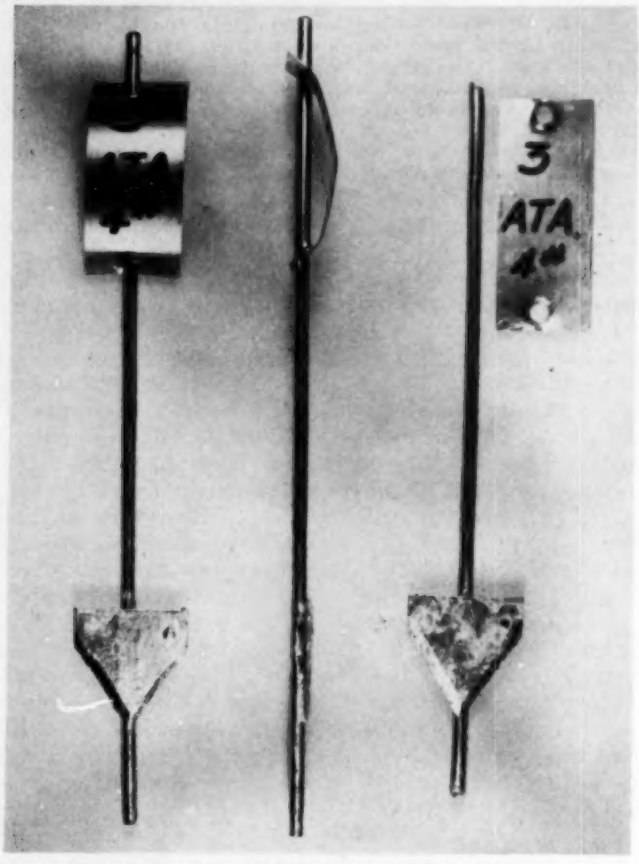

Figure 1. Metal plot stake designed for use in rocky soils.

[1]Contribution of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Washington Agricultural Experiment Stations cooperating.

[2]Agronomist and Agricultural Aid, respectively, Crops Research Division, Agricultural Research Service, Irrigation Experiment Station, Prosser, Washington.

these stakes are adapted for use in other types of soil also, especially those which are heavy and compact.

The stakes were made by spot-welding worn or discarded sections from a mower sickle to 18- or 20-inch lengths of ⅜-inch metal rod as illustrated in Figure 1. The point of the sections was positioned from 2½ to 3 inches from the bottom end of the rods. This permitted the stakes to be driven readily into the ground to depths of 4 to 6 inches and held in a desired position. To reduce cost for the rod, wheel spokes and brace rods from old machinery and army surplus rod stock were utilized as sources of supply.

For labeling purposes, tags 2½ x 6 or 3 x 6 inches were cut from light weight sheet metal (24 to 28 gauge). A ½-inch hole was drilled near each end and counter-pressed (dimpled) from opposite sides. By moderately flexing the resilient metal tags between thumb and forefinger, the upper ends of the rods were inserted readily through the holes. Back-spring prevented the mounted tags from slipping up or down on the rods. The rods and tags were coated with aluminum paint after which plot numbers and treatment designations were printed on the tags. If desired, the tags may be removed, changed, or replaced readily and conveniently without pulling the stakes.

News and Notes

Lloyd Sherwood accepted an appointment on the staff of Southern Illinois University, Carbondale, where he will be concerned with weed research and teaching. Sherwood was formerly employed by Monsanto Chemical Co., St. Louis, Missouri.

C. L. Foy is now in charge of weed control research in agronomic crops and is teaching a weed control course in the Department of Botany, University of California, Davis. He recently received the Ph.D. degree from the University of California.

Dr. C. G. McWhorter, formerly Research Assistant at Louisiana State University, has joined the staff at the Delta Experiment Station, Stoneville, Mississippi. He is employed by the Weeds Investigation Section and the Mississippi Agricultural Experiment Station.

Dr. R. D. Palmer, formerly Research Assistant at Louisiana State University, joined the staff of the Mississippi Agricultural Experiment Station at State College, Mississippi, February 1, 1959. Dr. Palmer will do basic physiological work as well as weed control in vegetable crops.

Leonard Hill, Ltd. announce that the next International Crop Protection and Pest Control Exhibition, organised by "World Crops", will be held from Wednesday, December 9 to Friday, December 11, 1959, at the Seymour Hall, St. Marylebone, London, W. 1. This date has been chosen in response to many requests that the Exhibition should be held in the winter. In fact this year's Exhibition will coincide with the Smithfield Show when thousands of farmers will be in London. The aim of the Exhibition is to cover

all aspects of crop protection and pest control. Further details can be obtained from "World Crops", Leonard Hill House, Eben Street, London N.W. 1.

The next meeting of the Weed Society of America, and also of the Western Weed Control Conference, will be held February 22–25, 1960, at the Cosmopolitan Hotel in Denver, Colorado. The Western Weed Control Conference will be the host conference for this meeting. Officers of the WSA are: A. S. Crafts, University of California, president; K. P. Buchholtz, University of Wisconsin, Madison, vice president; W. C. Shaw, Crops Research Division, ARS, USDA, Beltsville, Maryland, secretary; and W. C. Jacob, University of Illinois, Urbana, treasurer and business manager.

The next meeting of the North Central Weed Control Conference will be held December 9 and 10, 1959, at the Royal Alexandra Hotel in Winnipeg, Manitoba. The new officers of the Conference are J. Roe Foster, Canadian Department of Agriculture, President; L. G. Holm, University of Wisconsin, Vice-President; and John D. Furrer, University of Nebraska, Secretary-Treasurer.

At the 1958 meeting in Cincinnati 450 registered. The Proceedings, which this year includes the printed Research Reports, can be obtained from John D. Furrer, Department of Agronomy, University of Nebraska, Lincoln, Nebraska, for $4.00.

The 1960 meeting of the Northeastern Weed Control Conference will be held at the Hotel New Yorker, New York City, on January 6, 7, and 8. The officers for 1959 are L. G. Utter, The Diamond Alkali Company, President; E. M. Rahn, University of Delaware, Vice-President; D. A. Schallock, Rutgers University, Secretary-Treasurer.

The 1959 meeting on January 7, 8, and 9, also at the Hotel New Yorker, brought a record-breaking 615 registrants. The Proceedings of the Conference can be obtained from D. A. Schallock, Extension Weed Control Specialist, Rutgers University, New Brunswick, New Jersey, for $4.00 per copy. A registration and membership fee of $2.00 entitles the registrant to the Supplement to the Proceedings, two Newsletters, the call for papers, the advance program and the preliminary Coordinating Committee report.

The 1960 meeting of the Southern Weed Conference will be held at Biloxi, Mississippi, January 20, 21, and 22. The officers for 1959 are V. S. Searcy, Alabama Polytechnic Institute, President; Robert Darrow, Texas A & M College, Vice-President; and Walter K. Porter, Jr., Botany Department, Louisiana State University, Baton Rouge, Secretary-Treasurer. The 1959 Proceedings of the Southern Weed Conference can be obtained from Dr. Porter for $3.50.

BIBLIOGRAPHY OF WEED INVESTIGATIONS

for

July, August, September, 1958

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Agricultural Research Service

Crops Research Division
Weed Investigations Section

This bibliography of current literature on weeds and their control is issued as a service to those engaged in weed research in the United States and other countries. In issuing this bibliography the Crops Research Division, Agricultural Research Service, U.S.D.A. does not assume responsibility for the subject matter cited.

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